

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
18 November 2004 (18.11.2004)

PCT

(10) International Publication Number
WO 2004/099422 A2

(51) International Patent Classification⁷: **C12N 15/861**,
C07K 14/075, A61K 39/00, 48/00, C12N 5/10, C07K
14/19

(21) International Application Number:
PCT/US2004/009219

(22) International Filing Date: 24 March 2004 (24.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/459,000 28 March 2003 (28.03.2003) US
60/467,500 1 May 2003 (01.05.2003) US

(63) Related by continuation (CON) or continuation-in-part
(CIP) to earlier applications:
US 60/459,000 (CON)
Filed on 28 March 2003 (28.03.2003)
US 60/467,500 (CON)
Filed on 1 May 2003 (01.05.2003)

(71) Applicant (for all designated States except US): **THE
SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550
North Torrey Pines Road, TPC-8, La Jolla, CA 92037
(US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **VON SEGGERN,
Daniel, J.** [US/US]; 12557 Ruetten, Alliant #180, San
Diego, CA 92130 (US).

(74) Agents: **SEIDMAN, Stephanie, L.** et al.; Fish & Richard-
son P.C., 12390 El Camino Real, San Diego, CA 92130
(US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,

GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for the following desig-
nations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW,
BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ,
EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ,
OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA,
ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ,
SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ,
BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE,
BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,
IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
SN, TD, TG)

Published:

— without international search report and to be republished
upon receipt of that report
— with sequence listing part of description published sepa-
rately in electronic form and available upon request from
the International Bureau

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ADENOVIRUS PARTICLES WITH ENHANCED INFECTIVITY OF DENDRITIC CELLS AND PARTICLES WITH
DECREASED INFECTIVITY OF HEPATOCYTES

(57) Abstract: Provided are adenovirus vectors and the production of such vectors. In particular, adenoviruses with modified or
heterologous fiber proteins for targeting to dendritic cells are provided.



WO 2004/099422 A2

-1-

ADENOVIRUS PARTICLES WITH ENHANCED INFECTIVITY OF DENDRITIC CELLS AND PARTICLES WITH DECREASED INFECTIVITY OF HEPATOCYTES

Work described and claimed herein was supported by Department
5 of Defense Prostate Cancer Research Program DAMD17-01-1-0098
Department of Defense Breast Cancer Research Program DAMD17-01-1-0391. The government has certain rights in such subject matter.

RELATED APPLICATIONS

Benefit of priority is claimed to U.S. provisional application Serial
10 No. 60/459,000, filed March 28, 2003, entitled "DETARGETING OF
ADENOVIRAL PARTICLES AND USES THEREOF", to Daniel J. Von
Seggern, and to U.S. provisional application Serial No. 60/467,500, filed
May 1, 2003, entitled "PSEUDOTYPED ADENOVIRAL VECTORS WITH
ENHANCED INFECTIVITY TOWARDS DENDRITIC CELLS", to Daniel J.
15 Von Seggern.

This application also is related to U.S. application Serial No.
(attorney docket number 22908-1239), filed the same day herewith,
entitled "PSEUDOTYPED ADENOVIRAL VECTORS WITH ENHANCED
INFECTIVITY TOWARDS DENDRITIC CELLS," to Daniel J. Von Seggern.
20 This application also is related to U.S. application Serial No. 10/403,337,
filed March 27, 2003 and U.S. application Serial No. 10/351,890, filed
January 24, 2003, to Michael Kaleko, Glen R. Nemerow, Theodore Smith
and Susan C. Stevenson, entitled "FIBER SHAFT MODIFICATIONS FOR
EFFICIENT TARGETING". This application also is related to U.S.
25 provisional application Serial No. 60/350,388, filed January 24, 2002,
entitled "FIBER SHAFT MODIFICATIONS FOR EFFICIENT TARGETING," to
Susan C. Stevenson, Michael Kaleko, Theodore Smith and Glen R.
Nemerow, and to U.S. provisional application Serial No. 60/391,967,
filed June 26, 2002, entitled "FIBER SHAFT MODIFICATIONS FOR
30 EFFICIENT TARGETING," to Stevenson, Susan C., Kaleko, Michael,

-2-

Smith, Theodore and Nemerow, Glen R. This application also is related to International PCT application No. PCT/US03/02295, filed January 24, 2003, entitled "FIBER SHAFT MODIFICATIONS FOR EFFICIENT TARGETING," to Michael Kaleko, Glen R. Nemerow, Theodore Smith and
5 Susan C. Stevenson.

Where permitted, the subject matter of each of these applications, provisional applications and international applications is incorporated by reference herein.

FIELD OF INVENTION

10 The present invention generally relates to the field of adenoviral vectors and the production of such vectors. Targeted and detargeted adenoviral vectors are provided. In particular, adenoviral vectors targeted to dendritic cells are provided.

BACKGROUND

15 The immune system is designed to eradicate a large number of pathogens, as well as tumors, with minimal immunopathology. When the immune system becomes defective, however, numerous disease states result. Immunotherapy is an emerging treatment modality that seeks to harness the power of the human immune system to treat disease.
20 Immunotherapy is designed either to enhance the cellular immune response in subjects with diseases characterized by immunosuppression and/or to suppress the cellular immune response in subjects with diseases characterized by an overactive cellular immune response and/or to mount an immune response against pathogens or tumors. Improved
25 immunotherapeutic protocols are needed.

In addition, despite the extensive characterization of numerous infectious agents and the availability of vaccines, new vaccines are needed to protect against or ameliorate diseases, such as tuberculosis, malaria (Plebanski *et al.* (2002) *J. Clin. Invest.* 110:295-301), and a large

-3-

number of viruses including human immunodeficiency virus (HIV), herpes
simples virus (HSV), human papilloma virus (HPV), Epstein-Barr virus
(EBV), hepatitis C virus (HCV), respiratory syncytial virus (RSV),
parainfluenza viruses and human metapneumovirus (Letvin (2002) *J. Clin.*
5 *Invest. 110*:15-20; Whitley and Roizman (2002) *J. Clin. Invest. 110*:145-
151; Murphy and Collins (2002) *J. Clin. Invest. 110*:21-27), caused by
many clinically-relevant pathogens . Although vaccines have been
developed for influenza and anthrax, more effective vaccines to prevent
or reduce the severity of the diseases caused by these agents are needed
10 (see, *e.g.*, Palese and Garcia-Sastre (2002) *J. Clin. Invest. 110*:9-13;
Leppla *et al.* (2002) *J. Clin. Invest. 110*:141-144; Steinman and Pope
(2002) *J. Clin. Invest. 109*:1519-1526).

Vaccines and immunotherapy have been used to eliminate or a
wide variety of cancerous cells and tumors to thereby effect treatment of
15 cancer. Many human cancers are associated with the expression of
specific proteins, such as tumor antigens, thus providing a means of
identifying cancerous cells from normal cells, and providing a target for
immunotherapy. The immune system is capable of recognizing these
tumor antigens and eliciting an immune response directed against cells
20 displaying the tumor antigen (van der Bruggen *et al.* (1991) *Science*
254:1643-1647; Sahin *et al.* (1995) *Proc. Natl. Acad. Sci. U.S.A.*
92:11810-11813; Kaplan *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.*
95:7556-7561). The identification of these tumor antigens has led to the
development of vaccine and immunotherapeutic approaches for the
25 treatment of cancer (Scanlan and Jäger (2001) *Breast Cancer Res. 3*:95-
98; Yu and Restifo (2002) *J. Clin. Invest. 110*:28-94).

There, however, remain numerous challenges in the developement
of effective immunotherapies. These include, for example, a need for
(i) enhancing antibody and T cell-mediated immune memory,

-4-

- (ii) enhancing T cell responses (CD4+ T helper cells and CD8+ CTLs),
(iii) establishing mucosal immunity, which is important for vaccination
against many sexually transmitted diseases, (iv) development of vaccines
that can diminish the immune response, which is important for the
5 treatment of autoimmune diseases (Steinman and Pope (2002) *J. Clin.
Invest.* 109:1519-1526) and (v) others.

- Most, if not all, adenoviral vector-mediated gene therapy strategies
aim to transduce a specific tissue, such as a tumor or an organ, or a
specific cell type, cells as immune cells. Systemic delivery will require
10 ablation of the normal virus tropism as well as addition of new
specificities. Multiple interactions between adenoviral particles and the
host cell are required to promote efficient cell entry (Nemerow (2000)
Virology 274:1-4). An adenovirus entry pathway is believed to involve
two separate cell surface events. First, the adenoviral fiber knob
15 mediates attachment of the adenovirus particle to a target cell through a
high affinity interaction with a specific cell-surface receptor, which is the
coxsackie-adenovirus receptor (CAR) for most, but not all, serotypes of
adenovirus. A subsequent association of penton with cell surface
integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which act as co-receptors, potentiates virus
20 internalization. Although there are a plurality of adenoviral fiber
receptors, in addition to CAR, that interact with subgroup B (*e.g.*, Ad3)
and subgroup C (*e.g.*, Ad5) adenoviruses, both subgroups appear to
require interaction with integrins for internalization.

- The role of CAR interaction for *in vivo* gene transfer is not clear.
25 CAR ablation does not change biodistribution and toxicity of adenoviral
vectors *in vivo* (Alemany *et al.* (2001) *Gene Therapy* 8:1347-1353; U.S.
patent application No. 09/870,203, filed May 30, 2001, and published as
U.S. Published application No. 20020137213). Published studies have
described conflicting results (Alemany *et al.* (2001) *Gene Therapy*

-5-

8:1347-1353; Leissner *et al.* (2001) *Gene Therapy* 8:49-57; Einfeld *et al.* (2001) *J. Virology* 75:11284-11291). For example, it has been shown that vectors containing an S408E mutation in the Ad5 fiber AB loop yield efficient liver transduction in mice, despite having greatly reduced
5 transduction efficiencies on cells in culture (see, Leissner *et al.* (2001) *Gene Therapy* 8:49-57). In contrast, vectors containing a more extensive fiber AB loop mutation showed a 10-fold reduction in liver gene expression (see, Einfeld *et al.* (2001) *J. Virology* 75:11284-11291).

A doubly ablated adenovirus has been prepared by modifying the
10 CAR binding region in the fiber loop and the integrin binding region in the penton base (Einfeld *et al.* (2001) *J. Virology* 75:11284-11291). This doubly ablated adenovirus, lacking CAR and integrin interactions, was reported not only to lack *in vitro* transduction of various cell types but also to lack *in vivo* transduction of liver cells. Specifically, the doubly
15 ablated adenovirus was reported to have a 700-fold reduction in liver transduction when compared to the non-ablated adenovirus. These results, however, were not reproduced by others.

For many applications, the most clinically useful adenoviral vector would be deliverable systemically, such as into a peripheral vein, and
20 would be targeted to a desired location in the body or a desired cell type, and would not have undesirable side effects resulting from targeting to other locations. *In vivo* adenoviral vector targeting is a major goal in gene therapy and a significant effort has been focused on developing strategies to achieve this goal. Successful targeting strategies would direct the
25 entire vector dose to the appropriate site and would be likely to improve the safety profile of the vector by permitting the use of lower, less toxic vector doses, which potentially also can be less immunogenic. Thus, there is a need to develop adenoviruses that are fully detargeted *in vivo* for use as a base vector for producing redirected adenoviruses.

-6-

Therefore, among the objects herein, it is an object to provide detargeted adenoviral vectors, methods for preparation thereof, and uses thereof. Furthermore, it is in an object herein to provide immunotherapeutic methods and compositions therefor.

5 SUMMARY

Provided are immunotherapeutic methods and compositions that have immunotherapeutic activity. In particular, adenoviral vectors that deliver antigens to dendritic cells for processing and presentation to T cells are provided. Delivery of antigens to dendritic cells has preventive,
10 diagnostic and therapeutic applications.

Detargeted and fully detargeted adenoviral particles from serotype C, such as adenovirus 2 and adenovirus 5, adenovirus vectors from which such particles are produced, methods for preparation of the vectors and particles and uses of the vectors and particles are provided. Retargeted
15 particles also are provided.

The particles are detargeted from binding to certain native receptors (*e.g.*, coxsackie-adenovirus receptor (CAR) for Ad5 and Ad2), and can be targeted to receptors expressed on dendritic cells. In addition, among the viral particles provided are particles that do not bind to or
20 exhibit reduced binding to HSP (Heparin Sulfate Proteoglycans; also referred to as heparin sulfate glycosaminoglycans), and, hence, exhibit reduced or no binding to hepatocytes, which express HSPs.

Provided are the adenoviral particles and genomes encoding such particles and/or genomes (viral nucleic acid molecules), cell lines and
25 methods for producing such particles. In particular genomes that encode Ad5 particles or other type C viral particles that express fibers from adenovirus subgroup D or subgroup B, such as Ad19p, Ad30, Ad37, Ad16 and Ad35 (or that express modified fibers thereof) are provided. The fibers are modified to permit incorporation into the particle. The viral

-7-

particles provided herein exhibit reduced binding to hepatocytes and hence reduced liver toxicity.

Adenoviral particles that contain a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased and binding to heparin sulfate proteoglycans (HSP) and to CAR is reduced or eliminated compared to a particle that expresses its native fiber are provided. In these particles, the adenoviral (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and the fiber is from an adenovirus subgroup D, such as Ad19p. In another embodiment, the heterologous fiber is from Ad30. In other embodiments, the fiber is chimeric and comprises an N-terminal portion from a fiber of a subgroup C Ad virus; and the N-terminal portion is sufficient to increase incorporation into the particle compared to in its absence. For example, the fiber can be from an adenovirus Ad19p, Ad30, Ad37, Ad16 or Ad35 virus. The fiber protein can additionally include one or more further modifications that reduce or eliminate interaction of the resulting fiber with one or more cell surface proteins, such as CAR, in addition to HSP.

The adenoviral particle also can include a mutation in the CAR-binding region of the capsid and/or a mutation in the α_v integrin-binding region of the capsid, whereby binding to the integrin is eliminated or reduced. The CAR-binding region of the capsid that is modified can be on a fiber knob.

In some embodiments, the chimeric fiber contains at least a sufficient number of amino acids of Ad19p fiber set forth as SEQ ID No. 34 to target a particle to dendritic cells, and optionally to reduce or eliminate binding of the particle to HSP. For example, the Ad19p fiber is modified by replacing at least the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber. In other embodiments, the chimeric fiber contains at least a sufficient number of

-8-

amino acids of Ad30 fiber set forth as SEQ ID No. 36 to target a particle to dendritic cells, and optionally to reduce or eliminate binding of the particle to HSP. For example, the Ad30 fiber is modified by replacing at least the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17

5 amino acids of an Ad2 or Ad5 fiber.

Hence, also provided are methods for making and using the adenoviral particles that express the modified fibers and combinations of modified fibers and modified penton. With the fiber shaft modifications, particularly in combination with the fiber knob modifications and the

10 penton modifications, the adenovirus particles are ablated for binding to their natural cellular receptor(s), *i.e.*, they are detargeted. In addition, by selection of a subgroup D fiber, the resulting particles are targeted to dendritic cells. The particles also can be "retargeted" to a specific cell type through the addition of a ligand to the virus capsid, which causes
15 the virus to bind to and infect such cell. The ligand can be added, for example, through genetic modification of a capsid protein gene.

The nucleic acids, proteins, adenoviral particles and adenoviral vectors have a variety of uses. These include *in vivo* and *in vitro* uses to target nucleic acid to particular cells and tissues, for therapeutic

20 purposes, including gene therapy, and also for the identification and study of cell surface receptors and identification of modes of interaction of viruses with cells.

Nucleic acids encoding the capsid proteins, including the fibers are also provided. The nucleic acids can be provided as vectors, particularly
25 as adenovirus vectors. Many adenoviral vectors are known and can be modified as needed in accord with the description herein. Adenoviral vectors include, but are not limited to, early generation adenoviral vectors, such as E1-deleted vectors, gutless adenoviral vectors and replication-conditional adenoviral vectors, such as oncolytic adenoviral

-9-

vectors. The adenovirus vectors also can include heterologous nucleic acids that encode or provide products, such as tumor antigens and antigens from pathogens that induce an immunotherapeutic response whereby infection with such pathogen is prevented or the symptoms of infection reduced. Heterologous nucleic acid can encode a polypeptide or comprise or encode a regulatory sequence, such as a promoter or an RNA, including RNAi, small RNAs, other double-stranded RNAs, antisense RNA, and ribozymes. Promoters include, for example, constitutive and regulated promoters and tissue specific promoters, including tumor specific promoters. The promoter can be operably linked, for example, to a gene of an adenovirus essential for replication.

Cells containing the nucleic acid molecules and cells containing the vectors are also provided. Such cells include packaging cells. The cells can be prokaryotic or eukaryotic cells, including mammalian cells, such as primate cells, including human cells.

Also provided are adenoviral particles that contain the modified capsid proteins provided herein. The particles have increased tropism for dendritic cells, and also exhibit altered interaction or binding with HSP compared to particles that do not contain the modified capsid proteins. In addition to altered binding to HSP and dendritic cells, the particles can include further modifications, such as capsid proteins with altered interaction with other receptors as described above. In particular, the particles can have altered, typically reduced or eliminated, interaction with CAR, α_v integrin and/or other receptors. The mutations include mutations in the fiber knob, penton and hexon. Exemplary fiber knob mutations are mutations in the AB loop or CD loop, such as KO1 or KO12. Such mutations include, for example, PD1, KO1, KO12 and S* (see, *e.g.*, U.S. provisional application Serial No. 60/459,000, and copending U.S. application Serial No. 10/351,890). In addition, the particles can include

-10-

additional ligands for retargeting to selected receptors. The adenoviral particles can be from any serotype and subgroup.

- Methods for expressing heterologous nucleic acids in a cell are provided. In these methods an adenoviral vector provided herein is
- 5 transduced into a cell to deliver the nucleic acid and/or encoded products. Transduction can be effected *in vivo* or *in vitro* or *ex vivo*, and can be for a variety of purposes including study of gene expression and genetic therapy. The cells can be prokaryotic cells, but typically are eukaryotic cells, including mammalian cells, such as primate, including human cells.
- 10 The cells can be of a specific type, such as a tumor cell or a cell in a particular tissue.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a plasmid map for pSKO1.

Figure 2 is a plasmid map for pNDSQ3.1KO1.

- 15 Figures 3A-3C are plasmid maps of pAdmireRSVnBg (Fig. 3A), pSQ1 (Fig. 3B) and pSQ1KO12 (Fig. 3C).

Figure 4 is a plasmid map for pSQ1PD1.

Figures 5A-5B are plasmid maps of pSQ1FKO1PD1 (Fig. 5A) and pSQ1KO12PD1 (Fig. 5B).

- 20 Figure 6 shows *in vitro* transduction efficiency of A549 cells using adenoviral vectors containing fiber AB loop knob and/or penton, PD1 mutations. The following adenoviral vectors were used in these studies: Av1nBg, Av1nBgFKO1, referred to as FKO1, Av1nBgPD1, referred to as PD1, and Av1nBgFKO1PD1 that is referred to as FKO1PD1.

- 25 Figure 7A-7B shows *in vivo* adenoviral-mediated liver gene expression (Fig. 7A) and hexon DNA content (Fig. 7B) using adenoviral vectors containing fiber AB loop knob and/or penton, PD1 mutations. The following adenoviral vectors were used in these studies: Av1nBg, Av1nBgFKO1, referred to as FKO1, Av1nBgPD1, referred to as PD1,

-11-

Av1nBgFKO1PD1, referred to as FKO1PD1, Av1nBgKO12, referred to as KO12, and Av1nBgKO12PD1 that is referred to as KO12PD1.

Figure 8 is a plasmid map for pFBshuttle(EcoRI).

Figure 9 is a plasmid map for pSQ1HSP.

5 Figure 10 is a plasmid map for pSQ1HSPKO1.

Figure 11 is a plasmid map for pSQ1HSPPD1.

Figure 12 is a plasmid map for pSQ1HSPKO1PD1.

Figures 13A-13C show the transduction efficiency of A549 and HeLa cells using adenoviral vectors containing fiber shaft, knob and/or penton mutations. Fig. 13A shows the dose response for the transduction efficiency of A549 cells. Fig. 13B shows the transduction efficiency of HeLa cells at 2000 ppc. Figure 13C shows the competition analysis of adenoviral vectors containing fiber shaft mutations.

15 Figures 14A-14B shows the influence of fiber shaft mutations on *in vivo* adenoviral-mediated liver gene expression (Fig. 14A) and hexon DNA content (Fig. 14B).

Figures 15A-15B are plasmid maps of pSQ1HSPRGD (Fig. 15A) and pSQ1HSPKO1RGD (Fig. 15B).

20 Figure 16 shows that insertion of a RGD targeting ligand can restore transduction of the vectors containing the HSP binding shaft S* mutation.

Figures 17A-17B are plasmid maps of pSQ1AD35Fiber (Fig. 17A) and pSQ1Ad35FcRGD (Fig. 17B).

25 Figures 18A-18B are maps of plasmids encoding 35F chimeric fibers. Fig. 18A is a plasmid map of pSQ135T5H, and Fig. 18B is a plasmid map of pSQ15T35H.

Figure 19 shows the results of an *in vitro* analysis of Ad5 vectors containing Ad35 fibers and derivatives thereof.

-12-

Figure 20 shows the results of an *in vivo* analysis of Ad5 vectors containing Ad35 fibers and derivatives thereof.

Figures 21A-21B are plasmid maps of pSQ1Ad41sF (Fig. 21A) and pSQ1Ad41sFRGD (Fig. 21B).

5 Figure 22 shows the results of an *in vivo* analysis of Ad5 vectors containing Ad41 short fiber.

Figure 23 shows the *in vitro* analysis of Ad5 based vectors containing the Ad41 short fiber which has been re-engineered to contain a cRGD ligand in the HI loop.

10 Figure 24 shows enhanced transduction of AE1-2a cells with the Av3nBgFKO1 detargeted adenoviral vector using hexadimethrine bromide (HB), protamine sulfate (PS) and poly-lysine-RGD (K14) or the anti-penton-TNF α bifunctional protein (α pen-TNF).

15 Figure 25 shows ablation of HSP interaction decreases adenoviral-mediated gene transfer to other organs.

20 Figure 26 shows *in vivo* liver transduction with adenoviral vectors which encode for β -galactosidase and contain various mutations to the fiber and/or penton proteins. Results are plotted as percent transduction as compared to wild type. Two different methods for determining the level of transduction are shown for each vector.

Figure 27 shows the adenoviral vector biodistribution to the liver and tumor for the vectors containing the S*, KO1S*, and 41sF fibers.

DETAILED DESCRIPTION

- 25 A. DEFINITIONS
- B. Adenovirus-cell interactions
1. Fiber protein
2. Pseudotyping
- C. Dendritic cell targeting
- 30 1. Dendritic cells
2. Dendritic cell therapies
3. Targeting adenoviral particles to dendritic cells
- a. Fiber substitution
- b. Efficient targeting

-13-

- 4. Additional modifications
- D. Adenovirus vector detargeting
- E. Nucleic acids, adenoviral vectors and cells containing the nucleic acids and cells containing the vectors
 - 1. Preparation of viral particles
 - 2. Adenoviral vectors and particles
 - a. Gutless vectors
 - b. Oncolytic vectors
 - 3. Packaging
 - 4. Propagation and scale-up
- F. Adenovirus expression vector systems
 - 1. Nucleic acid gene expression cassettes
 - 2. Promoters
- G. Heterologous polynucleotides and therapeutic nucleic acids
- H. Formulation and administration
 - 1. Formulation
 - 2. Administration
- I. Diseases, disorders and therapeutic products
- J. Examples

20 A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information.

As used herein, the term "adenovirus" or "adenoviral particle" is used to include any and all viruses that can be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Depending upon the

-14-

context reference to "adenovirus" can include adenoviral vectors. There are at least 51 serotypes of adenovirus that are classified into several subgroups. For example, subgroup A includes adenovirus serotypes 12, 18, and 31. Subgroup C includes adenovirus serotypes 1, 2, 5, and 6.

5 Subgroup D includes adenovirus serotypes 8, 9, 10, 13, 15, 17, 19, 19p, 20, 22-30, 32, 33, 36-39, and 42-49. Subgroup E includes adenovirus serotype 4. Subgroup F includes adenovirus serotypes 40 and 41. These latter two serotypes have a long and a short fiber protein. Thus, as used herein an adenovirus or adenovirus particle is a packaged vector or
10 genome.

As used herein, "virus," "viral particle," "vector particle," "viral vector particle," and "virion" are used interchangeably to refer to infectious viral particles that are formed when, such as when a vector containing all or a part of a viral genome, is transduced into an
15 appropriate cell or cell line for the generation of such particles. The resulting viral particles have a variety of uses, including, but not limited to, transferring nucleic acids into cells either *in vitro* or *in vivo*. For purposes herein, the viruses are adenoviruses, including recombinant adenoviruses formed when an adenovirus vector, such as any provided
20 herein, is encapsulated in an adenovirus capsid. Thus, a viral particle is a packaged viral genome. An adenovirus viral particle is the minimal structural or functional unit of a virus. A virus can refer to a single particle, a stock of particles or a viral genome. The adenovirus (Ad) particle is relatively complex and may be resolved into various
25 substructures.

Included among adenoviruses and adenoviral particles are any and all viruses that can be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Thus, as used herein, "adenovirus" and

-15-

"adenovirus particle" refer to the virus itself and derivatives thereof and cover all serotypes and subtypes and naturally occurring and recombinant forms, except where indicated otherwise. Included are adenoviruses that infect human cells. Adenoviruses can be wildtype or can be modified in various ways known in the art or as disclosed herein. Such modifications include, but are not limited to, modifications to the adenovirus genome that is packaged in the particle in order to make an infectious virus. Exemplary modifications include deletions known in the art, such as deletions in one or more of the E1a, E1b, E2a, E2b, E3, or E4 coding regions. Other exemplary modifications include deletions of all of the coding regions of the adenoviral genome. Such adenoviruses are known as "gutless" adenoviruses. The terms also include replication-conditional adenoviruses, which are viruses that preferentially replicate in certain types of cells or tissues but to a lesser degree or not at all in other types. For example, among the adenoviral particles provided herein, are adenoviral particles that replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. These include the viruses disclosed in U.S. Patent No. 5,998,205 and U.S. Patent No. 5,801,029. Such viruses are sometimes referred to as "cytolytic" or "cytopathic" viruses (or vectors), and if they have such an effect on neoplastic cells, are referred to as "oncolytic" viruses (or vectors).

As used herein, the terms "vector," "polynucleotide vector," "polynucleotide vector construct," "nucleic acid vector construct," and "vector construct" are used interchangeably herein to mean any nucleic acid construct that can be used for gene transfer, as understood by those skilled in the art.

As used herein, the term "viral vector" is used according to its art-recognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and can be packaged into a

-16-

viral vector particle. The viral vector particles can be used for the purpose of transferring DNA, RNA or other nucleic acids into cells either in vitro or in vivo. Viral vectors include, but are not limited to, retroviral vectors, vaccinia vectors, lentiviral vectors, herpes virus vectors (e.g.,
5 HSV), baculoviral vectors, cytomegalovirus (CMV) vectors, papillomavirus vectors, simian virus (SV40) vectors, Sindbis vectors, Semliki Forest virus vectors, phage vectors, adenoviral vectors, and adeno-associated viral (AAV) vectors. Suitable viral vectors are described, for example, in U.S. Patent Nos. 6,057,155, 5,543,328 and 5,756,086. The vectors provided
10 herein are adenoviral vectors.

As used herein, "adenovirus vector" and "adenoviral vector" are used interchangeably and are well understood in the art to mean a polynucleotide containing all or a portion of an adenovirus genome. An adenoviral vector, refers to nucleic encoding a complete genome or a
15 modified genome or one that can be used to introduce heterologous nucleic acid when transferred into a cell, particularly when packaged as a particle. An adenoviral vector can be in any of several forms, including, but not limited to, naked DNA, DNA encapsulated in an adenovirus capsid, DNA packaged in another viral or viral-like form (such as herpes
20 simplex, and AAV), DNA encapsulated in liposomes, DNA complexed with polylysine, complexed with synthetic polycationic molecules, conjugated with transferrin, complexed with compounds such as PEG to immunologically "mask" the molecule and/or increase half-life, or conjugated to a non-viral protein.

25 As used herein, oncolytic adenoviruses refer to adenoviruses that replicate selectively in tumor cells

As used herein, a variety of vectors with different requirements and purposes are described. For example, one vector is used to deliver particular nucleic acid molecules into a packaging cell line for stable

-17-

integration into a chromosome. These types of vectors also are referred to as complementing plasmids. A further type of vector carries or delivers nucleic acid molecules in or into a cell line (*e.g.*, a packaging cell line) for the purpose of propagating viral vectors; hence, these vectors also can be referred to herein as delivery plasmids. A third "type" of vector is the vector that is in the form of a virus particle encapsulating a viral nucleic acid and that is comprised of the capsid modified as provided herein. Such vectors also can contain heterologous nucleic acid molecules encoding particular polypeptides, such as therapeutic polypeptides or regulatory proteins or regulatory sequences to specific cells or cell types in a subject in need of treatment.

As used herein, the term "motif" is used to refer to any set of amino acids forming part of a primary sequence of a protein, either contiguous or capable of being aligned to certain positions that are invariant or conserved, that is associated with a particular function. The motif can occur not only by virtue of the primary sequence, but also as a consequence of three-dimensional folding. For example, the adenovirus fiber is a trimer, hence the trimeric structure can contribute to formation of a motif. Alternatively, a motif can be considered as a domain of a protein, where domain is a region of a protein molecule delimited on the basis of function without knowledge of and relation to the molecular substructure, as, *e.g.*, the part of a protein molecule that binds to a receptor. As shown herein, the motif KKTK constitutes a consensus sequence for fiber shaft interaction with HSP.

As used herein, cell therapy is a method of treatment involving the administration of live cells. Adoptive immunotherapy is a treatment process involving removal of cells from a subject, the processing of the cells in some manner *ex-vivo* and the infusion of the processed cells into the same or different subject as a therapy.

-18-

As used herein, a cell therapeutic refers to the compositions of cells that are formulated as a drug whose active ingredient is wholly or in part a living cell.

As used herein, immune cells are the subset of blood cells known
5 as white blood cells, which include mononuclear cells such as lymphocytes, monocytes, macrophages and granulocytes.

As used herein, T-cells are lymphocytes that express the CD3 antigen.

As used herein, helper cells are CD4+ lymphocytes.

10 As used herein, regulatory cells are a subset of T-cells, most commonly CD4+ T-cells, that are capable of enhancing or suppressing an immune response. Regulatory immune cells regulate an immune response primarily by virtue of their cytokine secretion profile. Some regulatory immune cells also can act to enhance or suppress an immune response by
15 virtue of antigens expressed on their cell surface and mediate their effects through cell-to-cell contact. Th1 and Th2 cells are examples of regulatory cells.

As used herein, effector cells are immune cells that primarily act to eliminate tumors or pathogens through direct interaction, such as
20 phagocytosis, perforin and/or granzyme secretion, induction of apoptosis, etc. Effector cells generally require the support of regulatory cells to function and also act as the mediators of delayed type hypersensitivity reactions and cytotoxic functions. Examples of effector cells are B lymphocytes, macrophages, cytotoxic lymphocytes, LAK cells,
25 NK cells and neutrophils.

As used herein, a professional antigen presenting cells (APC) include dendritic cells, B-cells and macrophages.

As used herein, the term "bind" or "binding" is used to refer to the binding between a ligand and its receptor, such as the binding of the Ad5

-19-

knob domain with CAR (coxsackie-adenovirus receptor), with a K_d in the range of 10^{-2} to 10^{-15} mole/l, generally, 10^{-6} to 10^{-15} , 10^{-7} to 10^{-15} and typically 10^{-8} to 10^{-15} (and/or a K_a of 10^5 - 10^{12} , 10^7 - 10^{12} , 10^8 - 10^{12} l/mole).

As used herein, specific binding or selective binding means that the
5 binding of a particular ligand and one receptor interaction (k_a or K_{eq}) is at least 2-fold, generally, 5, 10, 50, 100 or more-fold, greater than for another receptor. A statement that a particular viral vector is targeted to a cell or tissue means that its affinity for such cell or tissue in a host or *in vitro* is at least about 2-fold, generally, 5, 10, 50, 100 or more-fold,
10 greater than for other cells and tissues in the host or under the *in vitro* conditions.

As used herein, the term "ablate" or "ablated" is used to refer to an adenovirus, adenoviral vector or adenoviral particle, in which the ability to bind to a particular cellular receptor is reduced or eliminated, generally
15 substantially eliminated (*i.e.*, reduced more than 10-fold, 100-fold or more) when compared to a corresponding wild-type adenovirus. An ablated adenovirus, adenoviral vector or adenoviral particle also is said to be detargeted, *i.e.*, the modified adenovirus, adenoviral vector or adenoviral particle does not possess the native tropism of the wild-type
20 adenovirus. The reduction or elimination of the ability of the mutated adenovirus fiber protein to bind a cellular receptor as compared to the corresponding wild-type fiber protein can be measured or assessed by comparing the transduction efficiency (gene transfer and expression of a marker gene) of an adenovirus particle containing the mutated fiber
25 protein compared to an adenovirus particle containing the wild-type fiber protein for cells having the cellular receptor.

As used herein, tropism with reference to an adenovirus refers to the selective infectivity or binding that is conferred on the particle by a capsid protein, such as the fiber protein and/or penton.

-20-

As used herein, "penton" or "penton complex" is used herein to designate a complex of penton base and fiber. The term "penton" can also be used to indicate penton base, as well as penton complex. The meaning of the term "penton" alone should be clear from the context

5 within which it is used.

As used herein, the term "substantially eliminated" refers to a transduction efficiency less than about 11% of the efficiency of the wild-type fiber containing virus on HeLa cells. The transduction efficiency on HeLa cells can be measured (see, *e.g.*, Example 1 of U.S. Patent

10 Application Serial No. 09/870,203 filed on May 30, 2001, and published as U.S. Published application No. 20020137213, and of International Patent Application No. PCT/EP01/06286 filed June 1, 2001, and published as WO 01/92299). Briefly, HeLa cells are infected with the

15 adenoviral vectors containing mutated fiber proteins to evaluate the effects of fiber amino acid mutations on CAR interaction and subsequent gene expression. Monolayers of HeLa cells in 12 well dishes are infected with, for example, 1000 particles per cell for 2 hours at 37° C in a total volume of, for example, 0.35 ml of the DMEM containing 2% FBS. The

20 infection medium is then aspirated from the monolayers and 1 ml of complete DMEM containing 10% FBS was added per well. The cells are incubated for an sufficient time, generally about 24 hours, to allow for β -galactosidase expression, which is measured by a chemiluminescence reporter assay and by histochemical staining with a chromogenic

25 substrate. The relative levels of β -galactosidase activity are determined using as suitable system, such as the Galacto-Light chemiluminescence reporter assay system (Tropix, Bedford, Mass.). Cell monolayers are washed with PBS and processed according to the manufacturer's protocol. The cell homogenate is transferred to a microfuge tube and centrifuged to remove cellular debris. Total protein concentration is

-21-

determined, such as by using the bicinchoninic acid(BCA) protein assay (Pierce, Inc., Rockford, Ill.) with bovine serum albumin as the assay standard. An aliquot of each sample is then incubated with the Tropix β -galactosidase substrate for 45 minutes in a 96 well plate. A

- 5 luminometer is used determine the relative light units (RLU) emitted per sample and then normalized for the amount of total protein in each sample (RLU/ug total protein). For the histochemical staining procedure, the cell monolayers are fixed with 0.5% glutaraldehyde in PBS, and then were incubated with a mixture of 1 mg of
- 10 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal) per ml, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide and 2 mM $MgCl_2$ in 0.5 ml of PBS. The monolayers are washed with PBS and the blue cells are visualized by light microscopy, such as with a Zeiss ID03 microscope. Generally, the efficiency is less than about 9%, and typically is less than
- 15 about 8%.

- As used herein, the phrase "reduce" or "reduction" refers to a change in the efficiency of transduction by the adenovirus containing the mutated or heterologous fiber as compared to the adenovirus containing the wild-type fiber to a level of about 75% or less of the wild-type on
- 20 HeLa cells. Generally, the change in efficiency is to a level of about 65% or less than wild-type. Typically it is about 55% or less. This system is able to rapidly analyze modified fiber proteins and/or modified penton proteins for desired tropism in the context of the viral particle.

- As used herein, the term "mutate" or "mutation" or similar terms
- 25 refers to the deletion, insertion or change of at least one amino acid in the protein of interest (*e.g.* the part of the fiber shaft region interacting with HSP). The amino acid can be changed by substitution or by modification in a way that derivatizes the amino acid.

-22-

As used herein, the term "polynucleotide" means a nucleic acid molecule, such as DNA or RNA, that encodes a polynucleotide. The molecule can include regulatory sequences, and is generally DNA. Such polynucleotides are prepared or obtained by techniques known by those skilled in the art in combination with the teachings contained therein.

As used herein, the term "viral vector" is used according to its art-recognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and can be packaged into a viral vector particle. The viral vector particles can be used, for example, for transferring DNA into cells either *in vitro* or *in vivo*.

As used herein, adenoviral genome is intended to include any adenoviral vector or any nucleic acid sequence comprising a modified fiber protein. All adenovirus serotypes are contemplated for use in the vectors and methods herein.

As used herein, a packaging cell line is a cell line that is able to package adenoviral genomes or modified genomes to produce viral particles. It can provide a missing gene product or its equivalent. Thus, packaging cells can provide complementing functions for the genes deleted in an adenoviral genome (*e.g.*, the nucleic acids encoding modified fiber proteins) and are able to package the adenoviral genomes into the adenovirus particle. The production of such particles require that the genome be replicated and that those proteins necessary for assembling an infectious virus are produced. The particles also can require certain proteins necessary for the maturation of the viral particle. Such proteins can be provided by the vector or by the packaging cell.

As used herein, detargeted adenoviral particles have ablated (reduced or eliminated) interaction with receptors with which native particles. It is understood that *in vivo* no particles are fully ablated such that they do not interact with any cells. Detargeted particles have

-23-

reduced, typically substantially reduced, or eliminated interaction with native receptors. For purposes herein, detargeted particles have reduced (2-fold, 5-fold, 10-fold, 100-fold or more) binding or virtually no binding to CAR or another native receptor. The particles still bind to cells, but the
5 types of cells and interactions are reduced.

As used herein, pseudotyping describes the production of adenoviral vectors having modified capsid protein or capsid proteins from a different serotype from the serotype of the vector itself. One example, is the production of an adenovirus 5 vector particle containing an Ad37 or
10 Ad35 fiber protein. This can be accomplished by producing the adenoviral vector in packaging cell lines expressing different fiber proteins.

As used herein, receptor refers to a biologically active molecule that specifically or selectively binds to (or with) other molecules. The
15 term "receptor protein" can be used to more specifically indicate the proteinaceous nature of a specific receptor.

As used herein, the term "heterologous polynucleotide" means a polynucleotide derived from a biological source other than an adenovirus or from an adenovirus of a different strain or can be a polynucleotide that
20 is in a different locus from wild-type virus. The heterologous polynucleotide can encode a polypeptide, such as a toxin or a therapeutic protein. The heterologous polynucleotide can contain regulatory regions, such as a promoter regions, such as a promoter active in specific cells or tissue, for example, tumor tissue as found in oncolytic adenoviruses.
25 Alternatively, the heterologous polynucleotide can encode a polypeptide and further contain a promoter region operably linked to the coding region.

-24-

As used herein, the term "cyclic RGD" (or cRGD) refers to any amino acid that binds to α_v integrins on the surface of cells and contains the sequence RGD (Arg-Gly-Asp).

As used herein, the KO mutations refer to mutations in fiber that
5 knock out binding to CAR. For example, a KO1 mutation refers to a mutation in the Ad5 fiber and corresponding mutations in other fiber proteins. In Ad5, this mutation results in a substitution of fiber amino acids 408 and 409, changing them from serine and proline to glutamic acid and alanine, respectively. As used herein, a KO12 mutation refers to
10 a mutation in the Ad5 fiber and corresponding mutations in other fiber proteins. In Ad5, this mutation is a four amino acid substitution as follows: R512S, A515G, E516G, and K517G. Other KO mutations can be identified empirically or are known to those of skill in the art.

As used herein, PD mutations refer to mutations in the penton gene
15 that ablate binding by the encoded to α_v integrin by replacing the RGD tripeptide. The PD1 mutation exemplified herein results in a substitution of amino acids 337 through 344 of the Ad5 penton protein, HAIRGDTF (SEQ ID NO. 49), with amino acids SRGYPYDVPDYAGTS (SEQ ID NO. 50), thereby replacing the RGD tripeptide.

20 As used herein, reference to an amino acid in an adenovirus protein or to a nucleotide in an adenovirus genome is with reference to Ad5, unless specified otherwise. Corresponding amino acids and nucleotides in other adenovirus strains and modified strains and in vectors can be identified by those of skill in the art. Thus, recitation of a mutation is
25 intended to encompass all adenovirus strains that possess a corresponding locus.

As used herein, tumor antigen refers to a cell surface protein expressed or located on the surface of tumor cells.

-25-

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered.

As used herein, a therapeutically effective product is a product that
5 is encoded by heterologous DNA that, upon introduction of the DNA into a host, a product is expressed that effectively ameliorates or eliminates the symptoms, manifestations of an inherited or acquired disease or that cures said disease.

As used herein, a subject is an animal, such as a mammal, typically
10 a human, including patients.

As used herein, genetic therapy involves the transfer of heterologous DNA to the certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which such therapy is sought. The DNA is introduced into the selected target cells in a
15 manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. Alternatively, the heterologous DNA may in some manner mediate expression of DNA that encodes the therapeutic product, it may encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a
20 therapeutic product. Genetic therapy may also be used to deliver nucleic acid encoding a gene product to replace a defective gene or supplement a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid may encode a therapeutic compound, such as a growth factor inhibitor thereof, or a tumor necrosis
25 factor or inhibitor thereof, such as a receptor therefor, that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. The heterologous DNA encoding the therapeutic product may be modified prior to

-26-

introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

As used herein, a therapeutic nucleic acid is a nucleic acid that encodes a therapeutic product. The product can be nucleic acid, such as
5 a regulatory sequence or gene, or can encode a protein that has a therapeutic activity or effect. For example, therapeutic nucleic acid can be a ribozyme, antisense, double-stranded RNA, a nucleic acid encoding a protein and others.

As used herein, "homologous" means about greater than 25%
10 nucleic acid sequence identity, such as 25%, 40%, 60%, 70%, 80%, 90% or 95%. If necessary the percentage homology will be specified. The terms "homology" and "identity" are often used interchangeably. In general, sequences are aligned so that the highest order match is obtained (see, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed.,
15 Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence*
20 *Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). By sequence identity, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties established by each supplier. Substantially
25 homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid or along at least about 70%, 80% or 90% of the full-length nucleic acid molecule of interest. Also contemplated are nucleic acid molecules that

-27-

contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two nucleic acid molecules have nucleotide sequences that are at least, for example, 80%, 85%, 90%, 95%, 96%, 97%, 98%
5 or 99% "identical" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1):387 (1984)), BLASTP, BLASTN, FASTA (Atschul,
10 S.F., *et al.*, *J Molec Biol* 215:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available
15 programs include, DNASTar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI)). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.*
20 (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1)
25 a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of

-28-

3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide.

5 As used herein, the term "at least 90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polynucleotide length of 100 amino acids are compared, no more than 10% (i.e., 10 out of 100)
10 of amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying
15 length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be
20 assessed readily, often without relying on software.

As used herein: stringency of hybridization in determining percentage mismatch is as follows:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
- 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
- 25 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, *e.g.*, Sambrook, E.F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see,

-29-

also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate- buffered 0.18 M NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by T_m , which is a function of the sodium ion concentration and

- 5 temperature ($T_m = 81.5^\circ \text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - 600/(\text{l})$), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

- It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not
10 limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA* 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 $\mu\text{g}/\text{ml}$ denatured
15 salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

- Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA, 10% (wt/vol) dextran sulfate, and $5-20 \times 10^6$
20 cpm ^{32}P -labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are blotted dry and
25 exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

-30-

By way of example and not way of limitation, procedures using conditions of moderate stringency include, for example, but are not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10^6 cpm ^{32}P -labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS.

By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 $\mu\text{g/ml}$ denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P -labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency which can be used are well known in the art.

The term substantially identical or substantially homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 60% or 70%, preferably means

-31-

at least 80%, 85% or more preferably at least 90%, and most preferably at least 95% identity.

As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently
5 unchanged so that the substantially identical product can be used in place of the product.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel
10 electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce
15 substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

The methods and preparation of products provided herein, unless
20 otherwise indicated, employ conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art (see, *e.g.*, Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold
25 Spring Harbor, NY); Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel *et al.* (1992) *Current Protocols in Molecular Biology*, Wiley and Sons, New York; Glover (1985) *DNA Cloning I and II*, Oxford Press; Anand (1992) *Techniques for the Analysis of Complex Genomes*

-32-

- (Academic Press); Guthrie and Fink (1991) *Guide to Yeast Genetics and Molecular Biology*, Academic Press; Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Jakoby and Pastan, eds. (1979) *Cell Culture. Methods in Enzymology* 58, Academic Press, Inc., Harcourt Brace Jovanovich, NY; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal (1984), *A Practical Guide To Molecular Cloning; Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Hogan *et al.* (1986) *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

B. Adenovirus-cell interactions

- The ability of different subgroups of adenovirus to interact with, or not interact with, specific cell types and/or particular receptors can be exploited to produce adenoviruses with desired specificity. For example, adenovirus can be modified such that they are able to more efficiently target specific cell types and/or tissues. Adenovirus serotypes also can be modified to reduce or eliminate their interactions with a natural receptor and thereby reduce or eliminate the interaction of adenovirus with a particular cell type and/or tissue. Thus, provided herein are modifications of the viral capsid that alter the interaction of an adenovirus with its natural receptors and/or cell types and modifications that target an adenovirus to interact with other receptors and/or cell types. In particular, modifications that result targeting to dendritic cells are

provided. Also provided are modifications that result in reduction or ablation of the interaction of an adenovirus, particularly *in vivo*, with other cell types.

- Different adenovirus serotypes infect different cell types, largely
- 5 because their fibers bind distinct receptors (Defer *et al.* (1990) *J. Virol.* 64:3661-3673; Stevenson *et al.* (1995) *J. Virol.* 69:2850-2857; Arnberg *et al.* (2000) *J. Virol.* 74:42-48). For subgroup C viruses (including Ad2 and Ad5), coxsackievirus and adenovirus receptor (CAR) serves as the cellular receptor (Tomko and Philipson (1997) *Proc. Natl. Acad. Sci.*
- 10 *U.S.A.* 94:3352-3356; Bergelson *et al.* (1997) *Science* 275:1320-1323). While adenoviruses from several other subgroups also bind CAR (Roelvink *et al.* (1998) *J. Virol.* 72:7909-7915), infection and competition studies indicate that they use other proteins as primary receptors (Arnberg *et al.* (2000) *J. Virol.* 74:42-48; Huang *et al.* (1999) *J. Virol.* 73:2798-2802;
- 15 Segerman *et al.* (2000) *J. Virol.* 74:1457-1467; Wu *et al.* (2001) *Virology* 279:78-89; Shayakhmetov *et al.* (2000) *J. Virol.* 74:2567-2584).

1. Fiber Protein

- The adenovirus fiber protein is a homotrimeric protein containing three polypeptides of 62 kDa. Ad fiber proteins are located at each of the
- 20 twelve icosahedral vertices of the viral particle (Chroboczek *et al.* (1995) *Curr. Top. Microbiol. Immunol.* 199:163-200). The sequences of the fiber gene from a variety of serotypes including adenovirus serotypes 2 (Ad2), Ad5, Ad3, Ad12, Ad35, Ad40, and Ad41 are known. There are at least 21 different fiber genes in Genbank. Sequence analysis of fiber
- 25 proteins from several different adenovirus serotypes (Hong *et al.* (1988) *Virology* 167:545-553; Kidd *et al.* (1990) *Virology* 179:139-150; Signäs *et al.* (1985) *J. Virol.* 53:672-678) and the crystal structure of Ad2 fiber (van Raaij *et al.* (1999) *Nature* 401:935-938) have identified three structural domains in the fiber. The N-terminal region of the fiber protein

-34-

interacts with the penton base proteins to anchor the fiber to the viral particle. The C-terminal knob region is responsible for mediating virus binding to host cells. These two regions are connected via a long, thin central shaft region, which contains a variable number of shaft repeats, each repeat being made up of 15 residues designated as a-o. The repeating domains of the fiber shaft are characterized by an invariant glycine or proline at position j and a conserved pattern of hydrophobic residues (van Raaij *et al.* (1999) *Nature* 401:935-938). A conserved stretch of amino acids which includes the sequence TLWT (SEQ ID No. 46) marks the boundary between the repeating units of beta structure in the shaft and the globular head domain. The number of shaft repeats in Ad fiber depends on the adenoviral serotype. For example, Ad2 and Ad5 fiber proteins include 22 shaft repeats, while Ad3 contains only 5 repeats (Chroboczek *et al.* (1995) *Curr. Top. Microbiol. Immunol.* 199:163-200).

The C-terminal fiber knob mediates attachment to CAR, which is a 46 kDa protein of the immunoglobulin superfamily that is found on many different cell types (Bergelson *et al.* (1997) *Science* 275:1320-1323). A crystal structure of the Ad12 fiber in complex with CAR demonstrates that sequences in the fiber knob, specifically the AB loop, interact with the first Ig-like domain of CAR (Bewley *et al.* (1999) *Science* 286:1579-1583). Following attachment to CAR, binding of the Ad penton base protein to α_v integrins enables internalization and penetration of the virus into the cell.

Adenovirus interactions with specific cell types are also influenced by the capacity to bind HSP. As noted, adenoviruses having fiber shafts that do not interact with HSP include (a) adenoviruses of subgroup B, *e.g.*, Ad3, Ad35, Ad7, Ad11, Ad16, Ad21, (b) adenoviruses of subgroup F, *e.g.*, Ad40 and Ad41, specifically the short fiber, and (c) adenoviruses of subgroup D, which includes adenovirus serotype 8, 9, 10, 13, 15, 17,

-35-

19, 20, 22-30, 32, 33, 36-39, and 42-49. Serotype 19 has variants. Ad19p is a nonpathogenic variant of Ad19 (Arnberg *et al.* (1998) *Virology* 227:239-244) while Ad19a, along with Ad8 and Ad37, are major causes of EKC. Ad19a and Ad37 have identical fiber proteins (Arnberg *et al.* 5 (1998) *Virology* 227:239-244) and have similar tropism *in vivo*.

2. Pseudotyping

Adenoviral vectors can be modified for targeting specific tissue and/or cell types through a variety of modifications, including modifications to the viral capsid, particularly to the fiber protein.

10 Modifications provided herein include, but are not limited to, pseudotyping of the viral particle with heterologous and/or chimeric fiber protein.

Fibers that use non-CAR receptors can direct infection of a variety of different cell types (Shayakhmetov *et al.* (2000) *J. Virol.* 74:2567-2584; Von Seggern *et al.* (2000) *J. Virol.* 74:354-362; Law and Davidson 15 (2002) *J. Virol.* 76:656-661; Havenga *et al.* (2002) *J. Virol.* 76:4612-4620; Gall *et al.* (1996) *J. Virol.* 70:2116-2123; Chillon *et al.* (1999) *J. Virol.* 73:2537-2540), thus providing a means for adenovirus vector targeting. Adenovirus packaging cell systems allow generation of viral particles with essentially any desired fiber protein by trans- 20 complementation of a fiber-deleted virus (Von Seggern *et al.* (2000) *J. Virol.* 74:354-362; Von Seggern *et al.* (1999) *J. Virol.* 73:1601-1608). This technology, referred to as psuedotyping, allows generation of targeted viral particles that can be used to study tropism *in vitro* and *in vivo*, as well as permitting construction and propagation of viruses whose 25 fibers do not bind to the producer cells normally used for Ad growth.

As described herein, psuedotyping can be used to identify fibers from subgroup D adenoviruses that confer enhanced infectivity of dendritic cells. Fiberless adenovirus vectors can be pseudotyped with fiber proteins from different serotypes to generate adenovirus particles

-36-

with heterologous fiber proteins. Pseudotyping can be accomplished, for example, by expression in cells that contain expression plasmids encoding the fibers for pseudotyping. These vectors and plasmids can be generated as described herein or by any method known to those of skill in the art.

Accordingly, provided herein are modified fibers for targeting and detargeting and methods of making such fiber proteins and adenoviruses containing the fiber proteins. Among the cell types provided herein for adenovirus targeting are dendritic cells.

10 C. Dendritic cell targeting

Dendritic cells have numerous physiological features that render them desirable targets for immunotherapeutic approaches. Dendritic cells pick up antigens and migrate from the tissues of the body to the lymphoid tissues. There these cells present the antigens in lymphoid organs by displaying a foreign epitope bound to an MHC protein and trigger humoral and cellular immune responses. Dendritic cells have the ability to distinguish different types of pathogens, such as viruses, bacteria, fungi, and switch on specifically targeted immune-response genes against them. They are antigen-presenting cells that stimulate T lymphocytes into attacking infection. Hence delivery of heterologous antigens for presentation by dendritic cells provides a means for triggering humoral and cellular immune responses against such antigens. Also as noted, expression of particular products in dendritic cells also can function to inhibit or decrease in inappropriate or undesirable immune response, such as occurs in allergies, autoimmune diseases and inflammatory responses.

1. Dendritic Cells

Dendritic cells (abbreviated DCs), which have a variety of important physiological features in the immune system, can serve as targets for immunotherapy and vaccine development. Dendritic cells play an

-37-

important role in establishing an immune response. Dendritic cells are found in T-cell rich areas of the lymphoid tissues where they present antigen to T cells to stimulate the adaptive immune response (Janeway and Travers (1997) Immunobiology: the immune system in health and
5 disease, third edition, Current Biology Ltd., New York, N.Y.).

As an antigen presenting cell, the role of the dendritic cell is to capture foreign and self antigens, process them into peptides, and present the peptides in the context of MHC (major histocompatibility complex) proteins to T lymphocytes. Dendritic cells are highly specialized and
10 efficient APCs and they control the magnitude, quantity, and memory of the adaptive immune responses that they trigger (Steinman and Pope (2002) *J. Clin. Invest.* 109:1519-1526). The T cells activated by dendritic cells presenting antigen include, T-helper CD4+ cells, particularly cells designated Th1, and CD8+ cytotoxic T lymphocytes
15 (CTLs). Activated Th1 cells produce IFN- γ and induce proliferation and antibody production of antigen-specific B lymphocytes. CTLs activated by dendritic cells kill cells displaying antigen (such as virus-infected cells) by releasing cytotoxic granules into the cell (see, *e.g.*, Steinman and Pope (2002) *J. Clin. Invest.* 109:1519-1526).

20 As noted, dendritic cells express high levels of MHC molecules for antigen presentation rendering them highly efficient APCs. In addition they also express a high level of co-stimulatory molecules, which are important for enhancing an immune response. Dendritic cells also produce a wide array of immunostimulatory cytokines (Scanlan and Jäger
25 (2001) *Breast Cancer Res.* 3:95-98) and there potentiate and participate in immune responses, and have been used as targets for vaccine development. Engineering of dendritic cells to express a tumor antigen has been pursued as an approach to tumor immunotherapy. For example, genetically modified dendritic cells that express particular antigens, such

-38-

as tumor antigens, can be used as vaccines. In addition, genetic therapy that targets such cells *in vivo* can be used to generate APCs *in vivo* in immunotherapeutic methods.

Dendritic cells also are capable of diminishing an immune response.

- 5 Dendritic cells can be exploited to aid in vaccination against autoimmunity, allergy and transplantation rejection, all of which result from an uncontrolled or unchecked immune response (Hawiger *et al.* (2001) *J. Exp. Med.* 194:769-779; Steinman *et al.* (2003) *Annual Rev. Immunol.* 21:685-711). For example, dendritic cells appear to be
- 10 important for peripheral T cell tolerance (see, *e.g.*, Steinman *et al.* (2000) *J. Exp. Med.* 191:411-416). Tolerance, or unresponsiveness to an antigen, is critical for avoidance of autoimmunity. Dendritic cells are capable of inducing significant antigen-specific tolerance in peripheral lymphoid tissues (Hawiger *et al.* (2001) *J. Exp. Med.* 194:769-779), and
- 15 also are capable of inducing tolerance to transplantation antigens (see, Fu *et al.* (1996) *Transplantation* 62:659-665) and contact allergens (see, Steinbrink *et al.* (1997) *J. Immunol.* 159:4772-4780).

- Thus, vaccine and immunotherapeutic strategies involving dendritic cells are important for the treatment of a variety of clinically
- 20 important autoimmune and related diseases, including systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, insulin-dependent diabetes mellitus and Graves' disease, as well as for vaccination or treatment of cancers and diseases caused by pathogens.

2. Dendritic Cell Therapies

- 25 Different methods for delivery of the antigen gene to dendritic cells have been explored, but these generally require *ex vivo* manipulation of cells, including transfection, and then infusion of the cells. This is complicated, expensive, and requires generation of patient-specific reagents.

-39-

Adenovirus can be used for dendritic cell therapies. The ability of adenovirus serotypes to infect specific cell types, such as dendritic cells, can in part be attributed to their interaction, or a lack of interaction, with CAR. For example, the requirement for high doses of Ad5 in dendritic cell
5 (DC) transduction can be explained by the lack of CAR expression on dendritic cells (Linette *et al.* (2000) *J. Immunol.* 164:3402-3412; Tillman *et al.* (1999) *J. Immunol.* 162:6378-6383). Several approaches have been used to improve DC infection by adenoviruses. A bispecific antibody (Ab) which bound to the fiber knob as well as to CD40 (which is
10 expressed on the surface of DCs) was used to target dendritic cells (Tillman *et al.* (1999) *J. Immunol.* 162:6378-6383). This study showed that the cells expressed sufficient α_v integrins for efficient infection by the DC-binding adenovirus. This approach requires that production of the viral vector and the antibody, and purification of the complex, in clinically
15 acceptable forms. This presents problems with scale-up and manufacturing, and negates many of the advantages of adenovirus vectors, most notably simple production and purification, as well as vector stability.

Therefore to overcome these limitations, provided herein are
20 adenoviral vectors that have been modified for efficiently targeting dendritic cells. The adenoviral vectors can be used for targeting such cells *in vivo* and *ex vivo* for immunotherapy and *in vitro* for studying dendritic cell function.

3. Targeting Adenoviral Particles to Dendritic Cells

25 Numerous studies have shown that adenovirus (Ad)-mediated delivery to dendritic cells can lead to anti-tumor response, but the Ad vectors generally used in gene therapy are based on a serotype (Ad5) that infects dendritic cells very inefficiently. After *in vivo* Ad administration, infection of a fairly small number of dendritic cells has been directly

-40-

demonstrated (Zhang *et al.* (2001) *Mol. Therapy* 3:697-707; Oberholzer *et al.* (2002) *J. Immunol.* 168:3412-3418; Jooss *et al.* (1998) *J. Virol.* 72:4212-4223) and appears to be largely responsible for the cellular immune response observed (Zhang *et al.* (2001) *Mol. Therapy* 3:697-707; 5 Jooss *et al.* (1998) *J. Virol.* 72:4212-4223). Since Ad5 infects dendritic cells poorly (Dietz *et al.* (1998) *Blood* 91:393-398; Wan *et al.* (1997) *Human Gene Ther.* 8:1355-1363; Jonuleit *et al.* (2000) *Gene Therapy* 7:249-254; Linette *et al.* (2000) *J. Immunol.* 164:3402-3412; Tillman *et al.* (1999) *J. Immunol.* 162:6378-6383) high multiplicities of infection are 10 required.

Most testing has been done using primary cultures of dendritic cells derived from peripheral blood or bone marrow by incubation with cytokines, usually GM-CSF and IL-4 (Inaba *et al.* (1998) Isolation of dendritic cells *In Current Protocols in Immunology*, John Wiley & Sons, 15 Inc. Philadelphia, 3.7.1-3.7.15). *Ex vivo* infection of dendritic cells, followed by re-infusion, has been found to generate effective anti-tumor responses (Wan *et al.* (1997) *Human Gene Ther.* 8:1355-1363; Linette *et al.* (2000) *J. Immunol.* 164:3402-3412; Inoue *et al.* (1999) *Immunol. Lett.* 70:77-81; Sonderbye *et al.* (1998) *Exp. Clin. Immunogenet.* 15:100- 20 111; Ranieri *et al.* (1999) *J. Virol.* 73:10416-10425; Ribas *et al.* (1997) *Cancer Res.* 57:2865-2869; Miller *et al.* (2000) *Human Gene Ther.* 11:53-65). *Ex vivo* infection is not the ideal means for vaccination and immunotherapy.

In addition, recombinant adenoviruses with fiber proteins from the 25 subgroup B viruses Ad16 and Ad35 have been found to have an increased ability to infect human dendritic cells (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620; Rea *et al.* (2001) *J. Immunol.* 166:5236-5244). Subgroup B viruses, however, appear to have a broad tropism. For example, they transduce a wide variety of cultured cell lines as well as

-41-

primary cells from a number of different tissue types (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620), evidencing such broad tropism. This apparent lack of cell-specificity (broad tropism) demonstrated by subgroup B indicates that pseudotyping Ad5 or Ad2 viruses with Ad subgroup B
5 fibers is not advantageous.

a. Fiber Substitution

It is shown herein that, contrary to reports in the literature, subgroup D viruses can target dendritic cells. Subgroup D viruses exhibit a narrower tropism than subgroup B viruses. It is shown herein that
10 fibers from certain non CAR-using Ad serotypes, particularly Ad subgroup D viruses, effectively target receptors on dendritic cells.

Modified adenovirus particles can be generated by substituting dendritic cell-tropic fibers, such as the Subgroup D fibers, or portions thereof in place of the Ad subgroup C (or other Ad subgroup, including B
15 and D, with a heterologous fiber), such as Ad5 or Ad2 fiber to produce degarteted (reduced binding to CAR, HSP) and retargeted (to dendritic cells) viral particles.

Any portion of the fiber can be replaced with a portion of a subgroup D fiber, so long as the portion of the subgroup D fiber confers targeting to
20 dendritic cells and the fiber assembles into the viral capsid. In one embodiment, the entire fiber protein is replaced with a subgroup D fiber. In another embodiment, the entire fiber, except for the N-terminus is replaced. For example, at least about 16 or 17 amino acids or more, up to about 60, 70, 80, 90, 100 or more amino acids of N-terminus of the
25 native fiber is retained to aid in the incorporation of the fiber into the native particle.

Included in the modified adenoviruses provided herein are those with fiber protein from subgroup B and D, including, but not limited to Ad19p, Ad37, Ad30, Ad8, Ad9, Ad10, Ad13, Ad15, Ad17, Ad19, Ad20,

-42-

Ad16, Ad35 and adenovirus serotypes 22-30, 32, 33, 36-39, and 42-49, expressed on adenoviral particles, particularly subgroup C particles.

Among the modified capsid proteins are provided herein are those which include fibers containing the sequence of amino acids set forth in any of
5 SEQ ID NOs. 32, 34, 36, 38 or 40; or a sequence of amino acids having 60%, 70%, 80%, 90%, 95% or greater sequence identity with a sequence of amino acids set forth in any of SEQ ID NOs. 32, 34, 36, 38 or 40; or a sequence of amino acids encoded by a sequence of
10 nucleotides that hybridizes under conditions of high stringency along at least 70%, at least 80% or at least 90% of its length to a sequence of nucleotides that encodes a sequence of amino acids set forth in any of SEQ ID NOs. 32, 34, 36, 38 or 40. The fiber proteins can be modified, such as described herein, by replacement of the N-terminus to facilitate incorporation into the viral particle of a different subgroup, particularly
15 subgroup C. Such modification is generally inclusion of at least 16 or 17 amino acids up to about 60 or 61 or more contiguous amino acids from the N-terminus of the native fiber such that dendritic cell targeting is introduced.

In one exemplary embodiment, a packaging cell strategy is used to
20 produce particles of a fiber-deleted Ad5 vector containing fiber proteins from Ads of subgroup B (Ad3, Ad16, Ad35), subgroup C (Ad5), and subgroup D (Ad19p, Ad30, Ad37). Nucleotide and amino acid sequences of Ad fibers are set forth in SEQ ID NOs. 41-44 (exemplary chimeric fibers) and 31-40 (exemplary wild type fibers that can be modified by
25 replacement of the N-terminus). The resulting particles exhibit significant differences in dendritic cells tropism as demonstrated by their ability to infect primary murine bone marrow-derived DC *in vitro*. Furthermore, the particles pseudotyped with the subgroup D particles efficiently and specifically target dendritic cells. As described in the herein (see *e.g.*, the

-43-

Examples) subgroup B fibers appear to bind to receptors distinct from and more ubiquitously expressed than those bound by subgroup D fibers.

While particles with the Ad5 fiber infect dendritic cells rather poorly, vector particles pseudotyped with subgroup D fibers or portions thereof, such as the Ad19p and Ad37, were particularly effective. Thus, adenovirus particles, particularly subgroup C particles, modified to express all or a portion of a subgroup D fiber, efficiently target dendritic cells and can be used to deliver heterologous nucleic acids to such cells *in vivo* and *ex vivo*. In addition, such particles have reduced binding to HSP-expressing cells, such as hepatocytes and to CAR-expressing cells compared to unmodified subgroup B viral particles.

b. Efficient Targeting

Provided herein are recombinant adenoviruses with a limited tropism that target dendritic cells. The recombinant adenoviruses can be used for gene therapy and/or vaccination approaches. Administration can be effected *in vivo*, such as systemically, or *ex vivo* by contacting cells enriched for or containing dendritic cells.

The recombinant adenoviruses provided herein have a variety of advantageous properties. The particles provided herein more efficiently infect dendritic cells than Ad5 particles or Ad5 particles that express subgroup B fibers, and hence are more immunogenic following direct *in vivo* administration. The vectors provided herein that efficiently target dendritic cells permit not only *ex vivo* delivery, but direct *in vivo* administration, thereby eliminating the need for removal of cells, *ex vivo* cell culture, and infusion.

4. Additional Modifications

The modified adenoviruses provided herein not only exhibit

-44-

improved tropism for dendritic cells, but also reduced binding to HSP, which is expressed on liver cells. The modified particles can be further modified to be detargeted from CAR, HSP, α_v integrin, or any other native receptors, by any of the capsid mutations described below or well known to those of skill in the art.

The vectors provided herein also can be modified by including a RGD peptide in the fiber protein. It has been shown (see, *e.g.*, Okada *et al.* (*Cancer Res.* 61:7913-7919 (2001))) that incorporation of an RGD peptide into the fiber protein increased infection of a murine DC line approximately two-fold. The cell line/Ad system was then used to evaluate anti-tumor responses in a mouse tumor xenograft model. When DCs were infected *ex vivo* using equal particle numbers of the wild type or modified vectors and then re-infused into mice, the modified vector was able to stimulate a significantly better immune response against the model antigen.

The particles provided herein also can be further modified by inclusion of heterologous nucleic acid that provides a therapeutic product, and formulated for administration as vaccines. The adenovirus particles and vectors can deliver heterologous nucleic acids to dendritic cells to alter dendritic cell antigen presentation, cytokine production and other dendritic cell functions.

D. Adenovirus vector detargeting

Described below are modifications of the viral capsid that ablate the interaction of an adenovirus with its natural receptors. In particular, fiber modifications that result in ablation of the interaction of an adenovirus with HSP are described. These fiber modifications can be combined with other capsid protein modifications, such as other fiber modifications and/or penton and/or hexon modifications, to fully ablate viral interactions with natural receptors, when expressed on a viral particle. The

-45-

modification should not disrupt trimer formation or transport of fiber into the nucleus. The entire fiber of a serotype that binds to HSP can be replaced with all or a portion of a fiber that does not bind to HSP.

Generally in such instances, the N-terminus of the replacing fiber is

- 5 modified to resemble or to be identical to the replaced fiber to improve its incorporation into the viral particle. The number of amino acids at the N-terminus required can be empirically determined, but is typically between about 5-20, 10-17, 10-20, 10-50, 10-70, 10-100, amino acids, more amino acids can be included if convenient. The precise number also can
- 10 be based upon the presence of convenient restriction sites in the encoding nucleic acid and other such considerations. Generally at least about 5-20, such as 16, 17, or 18, amino acids are required.

The adenovirus fiber protein is a major determinant of adenovirus tropism (Gall *et al.* (1996) *J. Virol.* 70:2116-2123; Stevenson *et al.*

- 15 (1995) *J. Virol.* 69:2850-2857). Dogma in the field has been that adenoviral entry occurs via binding to CAR and integrins. This is underscored by published data (Einfeld *et al.* (2001) *J. Virology* 75:11284-11291). The published are not the predominant ones that act *in vivo*. The dominant entry pathway for hepatocytes *in vivo* involves a
- 20 mechanism mediated by the fiber shaft, such as Ad5 shaft, through heparin sulfate proteoglycans binding (see, published U.S. application Nos. 2004-0002060 and 2003-0215948).

Elimination of this binding eliminates entry via HSP binding, such as in hepatocytes. Adenoviral fiber shaft modifications that ablate viral

- 25 interaction with HSP are described in the Examples below and in published U.S. application Nos. 2004-0002060 and 2003-0215948. Thus, efficient detargeting of adenovirus *in vivo* can be achieved with appropriately designed fiber proteins. Suitable modifications, such as described herein, can be made with respect to any adenovirus in which

-46-

the wild-type interacts with HSP. The ability of an adenoviral vector to interact with HSP is modified by replacing the fiber protein or at least the binding portion thereof with a fiber (or corresponding portion thereof) that does not bind to HSP thereby reducing or eliminating binding to HSP.

- 5 This reduction or elimination of HSP binding can be manifested *in vivo* as reduced or eliminated transduction of liver cells in animals to whom the resulting viral particles are administered compared to the unmodified particle. Modifications include insertions, deletions, individual amino acid mutations and other mutations that alter the structure of the fiber shaft
- 10 such that the HSP binding of the modified fiber protein is ablated when compared to the HSP binding of the wild-type fiber protein.

- An adenoviral fiber protein is modified by mutating one or more of the amino acids that interact with HSP. For example, the HSP binding motif of the modified fiber protein is no longer able to interact with HSP
- 15 on the cell surface, thus ablating the viral interaction with HSP. For example, the adenoviral fiber is from a subgroup C adenovirus. Binding to HSP can be eliminated or reduced by mutating the fiber shaft in order to modify the ability of the HSP binding motif, which is, for example, KKTK sequence (SEQ ID NO. 45) located between amino acid residues 91 to 94
- 20 in the Ad5 fiber (SEQ ID NO. 2), to interact with HSP. The fiber proteins are modified by chemical and biological techniques known to those skilled in the art, such as site directed mutagenesis of nucleic acid encoding the fiber or other techniques as illustrated herein.

- In another aspect of this embodiment, the ability of a fiber to
- 25 interact with HSP is modified by replacing the wild-type fiber shaft with a fiber shaft, or portion thereof, of an adenovirus that does not interact with HSP to produce chimeric fiber proteins. The portion is sufficient to reduce or eliminate interaction with HSP. Examples of adenoviruses having fiber shafts that do not interact with HSP include (a) adenoviruses

-47-

of subgroup B, such as, but are not limited to, Ad3, Ad7, Ad11, Ad16, Ad21, Ad34 and Ad35 which do not have interaction with HSP, (b) adenoviruses of subgroup F, such as, but are not limited to, Ad40 and Ad41, specifically the short fiber, and (c) adenoviruses of subgroup D, such as but are not limited to, Ad19p, Ad30, Ad37 and Ad46.

In another embodiment, adenoviral fiber shaft modifications and/or pseudotyped fibers that ablate viral interaction with HSP in combination with adenoviral fiber knob modifications that ablate viral interactions with CAR are provided. Suitable adenoviral fiber modifications include the fiber knob modifications described in the Examples below and modifications known to those of skill in the art (see published U.S. application Nos. 2004-002060 and 2003-0215948; see, also, US. Patent Application Serial No. 09/870,203, filed on May 30, 2001, published as U.S. Published application No. 20020137213, and International Patent Application No. PCT/EP01/06286, filed on June 1, 2001, published as WO 01/92299). Modifications of the fiber include mutations of at least one amino acid in the CD loop of a wild-type fiber protein of an adenovirus from subgroup C (such as, *e.g.*, Ad2 or Ad5), subgroup D (such as, *e.g.*, Ad19p, Ad30 or Ad37), subgroup E, or the long wild-type fiber of an adenovirus from subgroup F, whereby the ability of a fiber protein to bind to CAR is reduced or substantially eliminated. The fiber proteins with ablated CAR interaction are modified by chemical and biological techniques known to those skilled in the art and as described herein.

Alternatively, adenoviral fiber modifications are made by replacing the wild-type fiber knob with a fiber knob of an adenovirus that does not interact with CAR. The fiber protein also will be selected so that it does not interact with HSP. Examples of adenoviruses having fiber knobs that do not interact with CAR include (a) adenoviruses of subgroup B, *e.g.*,

-48-

Ad3, Ad7, Ad11, Ad16, Ad21, Ad34, Ad35; and (b) adenoviruses of subgroup F, *e.g.*, Ad40 and Ad41, specifically the short fiber.

In another embodiment, adenoviral fiber shaft modifications and/or pseudotyped fibers that ablate viral interaction with HSP in combination
5 with penton modifications that ablate viral interactions with α_v integrins are provided. Suitable adenoviral penton modifications include the penton modifications, which are well known to those of skill in the art (see, *e.g.*, U.S. Patent No. 5,731,190; see, also Einfeld *et al.* (2001) *J. Virology* 75:11284-11291; and Bai *et al.* (1993) *J. Virology* 67:5198-5205).

10 For example, penton interaction with α_v integrins can be ablated (reduced or eliminated) by substitution of the RGD tripeptide motif, required for α_v interaction, in penton with a different tripeptide that does not interact with an α_v integrin. The penton proteins with ablated α_v integrin interactions are modified by chemical and biological techniques
15 known to those skilled in the art (see, *e.g.*, described U.S. Patent No. 6,731,190 and as illustrated herein).

Also provided are adenoviral fiber shaft modifications or pseudotyped fibers that ablate viral interaction with HSP in combination with adenoviral fiber knob modifications that ablate viral interactions with
20 CAR and with penton modifications that ablate viral interactions with α_v integrins. These modifications are described above and prepared using chemical and biological techniques known to those skilled in the art and as illustrated herein.

Preparation of fibers modified to eliminate or reduce HSP
25 interactions and fibers modified to alter interactions with other receptors and cell surface proteins, such as CAR and/or α_v integrin, also is described in the Examples below. The nucleic acid and/or amino acid sequences of exemplary modified fibers, whose construction are described below) are set forth as SEQ ID NOs. 3-30 as follows:

-49-

SEQ ID NOs. 3 and 4 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO1, where 5F refers to adenovirus 5 fiber, KO1 is an exemplary mutation of the CAR interaction site described herein;

- 5 SEQ ID NOs. 5 and 6 set forth the encoding nucleotide sequence and amino acid sequence of the modified ber designated 5FKO1RGD, which further includes an RGD ligand to demonstrate retargeting;

- SEQ ID NOs. 7 and 8 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO12, where
10 5F refers to adenovirus 5 fiber, KO12 is another exemplary mutation of the CAR interaction site described herein;

- SEQ ID NOs. 9 and 10 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5F S* nuc, where 5F refers to adenovirus 5 fiber, S* is an exemplary mutation of the
15 shaft that alters binding to HSP;

 SEQ ID NOs. 11 and 12 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5F S*RGD nuc, which further includes an RGD ligand;

- SEQ ID NOs. 13 and 14 set forth the encoding nucleotide sequence
20 and amino acid sequence of the modified ber designated 5FKO1S*, which contain the KO1 and S* mutations;

 SEQ ID NOs. 15 and 16 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5FKO1S*RGD, which further includes an RGD ligand;

- 25 SEQ ID NOs. 17 and 18 set forth the encoding nucleotide sequence and amino acid sequence of a Ad35 fiber;

 SEQ ID NOs. 19 and 20 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 35FRGD, which is 35F fiber with an RGD ligand;

-50-

SEQ ID NOs. 21 and 22 set forth the encoding nucleotide sequence and amino acid sequence of a Ad41 short fiber;

SEQ ID NOs. 23 and 24 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 41sFRGD,

5 which is 41F short fiber with an RGD ligand;

SEQ ID NOs. 25 and 26 set forth the encoding nucleotide sequence and amino acid sequence of Ad5 penton;

SEQ ID NOs. 27 and 28 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 5TS35H, which
10 is a chimeric fiber in which an Ad5 fiber tail and shaft regions (5TS; amino acids 1 to 403) are connected to an Ad35 fiber head region (35H; amino acids 137 to 323) to form the 5TS35H chimera; and

SEQ ID NOs. 29 and 30 set forth the encoding nucleotide sequence and amino acid sequence of the modified fiber designated 35TS5H, which
15 is a chimeric fiber in which an Ad35 fiber tail and shaft regions (35TS; amino acids 1 to 136) are connected to an Ad5 fiber head region (5H; amino acids 404 to 581) to form the 35TS5H chimera.

The modified fibers are displayed on virus particles by modifying the fiber protein and optionally additional proteins. This can be achieved
20 by preparing adenoviral vectors that express the modified capsid proteins and produce particles with modified fibers, or by packaging adenoviral vectors, particularly those that do not encode one or more capsid proteins in appropriate packaging lines. Hence, as discussed in detail below, adenoviral vectors and viral particles with modified fibers that do not bind
25 to HSP are provided.

-51-

Retargeting detargeted fibers

The viral particles that are detargeted as described above, can be retargeted to selected cells and/or tissues by inclusion of an appropriate targeting ligand in the capsid. The ligand can be included in any of the capsid proteins, such as fiber, hexon and penton. Loci for inclusion of nucleic acid encoding a targeting ligand is known to those of skill in the art for a variety of adenovirus serotypes; if necessary appropriate loci and other parameters can be empirically determined.

The ligand can be produced as a fusion by inclusion of the coding sequences in the nucleic acid encoding a capsid protein, or chemically conjugated, such as via ionic, covalent or other interactions, to the capsid or bound to the capsid (*e.g.*, by Ab-ligand fusion, where Ab binds capsid protein; or by disulfide bonding or other crosslinking moieties or chemistries).

Thus, for example, a modified fiber nucleic acid also can include sequences of nucleotides that encode a targeting ligand to produce viral particles that include a targeting ligand in the capsid. Targeting ligand and methods for including such ligands in viral capsids are well known. For example, inclusion of targeting ligands in fiber proteins is described in U.S. Patent Nos. 5,543,328 and 5,756,086 and in U.S. Patent Application Serial No. 09/870,203, published as U.S. Published application No. 20020137213, and International Patent Application No. PCT/EP01/06286, published as WO 01/92299. For different serotypes and strains of adenoviruses, loci for insertion of targeting ligands can be empirically determined. For different serotypes and strains, such loci can vary.

Because the adenovirus fiber has a trimeric structure, the ligand can be selected or designed to have a trimeric structure so that up to three molecules of the ligand are present for each mature fiber. Such

-52-

ligands can be incorporated into the fiber protein using methods known in the art (see, *e.g.*, U.S. Patent No. 5,756,086). Instead of the fiber, the targeting ligand can be included in the penton or hexon proteins.

Inclusion of targeting ligands in penton (see for example, in U.S. Patent
5 Nos. 5,731,190 and 5,965,431) and in hexon (see for example, in U.S. Patent No. 5,965,541) is known.

In one exemplary embodiment, the ligand is included in a fiber protein, which is a fiber protein mutated as described herein. The targeting ligand can be included, for example, within the HI loop of the
10 fiber protein. Any ligand that can fit in the HI loop and still provide a functional virus is contemplated herein. Such ligands can be as long as or longer than 80-100 amino acids (see, *e.g.*, Belousova *et al.* (2002) *J. Virol.* 76:8621-8631). Such ligands are added by techniques known in the art (see, *e.g.*, published International Patent Application publication
15 No. WO 99/39734 and U.S. Patent Application No. 09/482,682). Other ligands can be discovered through techniques known to those skilled in the art. Some non-limiting examples of these techniques include phage display libraries or by screening other types of libraries. Such ligands include any that target dendritic cells.

20 Targeting ligands include any chemical moiety that preferentially directs an adenoviral particle to a desired cell type and/or tissue, such as a dendritic cell. The categories of such ligands include, but are not limited to, peptides, polypeptides, single chain antibodies, and multimeric proteins. Specific ligands include the TNF superfamily of ligands which
25 include tumor necrosis factors (or TNF's) such as, for example, TNF α and TNF β , lymphotoxins (LT), such as LT- α and LT- β , Fas ligand which binds to Fas antigen; CD40 ligand, which binds to the CD40 receptor of B-lymphocytes; CD30 ligand, which binds to the CD30 receptor of neoplastic cells of Hodgkin's lymphoma; CD27 ligand, NGF ligand, and

-53-

OX-40 ligand; transferrin, which binds to the transferrin receptor located on tumor cells, activated T -cells, and neural tissue cells; ApoB, which binds to the LDL receptor of liver cells; alpha-2-macroglobulin, which binds to the LRP receptor of liver cells; alpha-I acid glycoprotein, which
5 binds to the asialoglycoprotein receptor of liver; mannose-containing peptides, which bind to the mannose receptor of macrophages; sialyl-Lewis-X antigen-containing peptides, which bind to the ELAM-I receptor of activated endothelial cells; CD34 ligand, which binds to the CD34 receptor of hematopoietic progenitor cells; ICAM-I, which binds to
10 the LFA-I (CD11b/CD18) receptor of lymphocytes, or to the Mac-I (CD11a/CD18) receptor of macrophages; M-CSF, which binds to the c-fms receptor of spleen and bone marrow macrophages; circumsporozoite protein, which binds to hepatic Plasmodium falciparum receptor of liver cells; VLA-4, which binds to the VCAM-I receptor of activated
15 endothelial cells; HIV gp120 and Class II MHC antigen, which bind to the CD4 receptor of T -helper cells; the LDL receptor binding region of the apolipoprotein E (ApoE) molecule; colony stimulating factor, or CSF, which binds to the CSF receptor; insulin-like growth factors, such as IGF-I and IGF-II, which bind to the IGF-I and IGF-II receptors, respectively;
20 Interleukins 1 through 14, which bind to the Interleukin 1 through 14 receptors, respectively; the Fv antigen-binding domain of an immunoglobulin; gelatinase (MMP) inhibitor; bombesin, gastrin-releasing peptide; substance P; somatostatin; luteinizing hormone releasing hormone (LHRH); vasoactive peptide (VIP); gastrin; melanocyte
25 stimulating hormone (MSH); cyclic RGD peptide and any other ligand or cell surface protein-binding (or targeting) molecule. Such ligands can be

-54-

advantageously employed with the Ad5 particles pseudotyped with subgroup D adenovirus fiber, such as, for example, Ad19p, Ad30 or Ad37 fiber.

5 E. Nucleic acids, adenoviral vectors and cells containing the nucleic acids and cells containing the vectors

Also provided are polynucleotides that encode modified, including chimeric and/or heterologous, capsid proteins and that encode vectors for preparation of adenovirus that express modified capsid proteins provided herein. The sequences of the wild-type adenovirus proteins from many
10 different adenovirus serotypes are well known in the art and are modified as described herein or by any suitable method.

Also provided are vectors including the polynucleotides provided herein. Such vectors include partial or complete adenoviral genomes and plasmids. Such vectors are constructed by techniques known to those
15 skilled in the art and as illustrated herein. Also provided are adenoviral vectors modified by replacing whole fiber protein, or portions thereof, with the fiber proteins, or appropriate portions thereof, from an adenovirus of a different serotype that more efficiently targets dendritic cells. Adenoviruses that target dendritic cells can be identified by using
20 the methods described herein. Their fiber-encoding genes can then be used to pseudotype viruses, such as Ad5 or Ad2 and infection and gene delivery of adenoviruses with the heterologous or chimeric fibers can be detected. Among the adenoviral vectors provided herein are those of subgroup C, which include Ad2 and Ad5, in which the nucleic acid
25 encoding the fiber knob and a portion or all of the fiber shaft domain is replaced with nucleic acid encoding fiber or an appropriate portion thereof from a subgroup D adenovirus, such as Ad19p, Ad30 or Ad37.

Thus, adenoviral fiber modifications or substitutions can be made in viral particles by replacing the entire fiber protein, or a portion thereof,

-55-

with the fiber protein of an adenovirus that more efficiently binds to receptors on dendritic cells. Generally the heterologous adenovirus fiber is from a subgroup D adenovirus, such as Ad19p, Ad30 or Ad37.

Adenoviral vectors of subgroup C, such as Ad2 and Ad5, having a
5 replaced fiber knob are prepared using techniques well known in the art and as illustrated herein.

In particular, as exemplified herein, the nucleic acid and/or amino acid sequences of exemplary heterologous and/or modified fibers for dendritic cell targeting are set forth as SEQ ID NOs. 31-44 as follows:

10 SEQ ID NOs. 31 and 32 set forth the encoding nucleotide sequence and amino acid sequence of Ad37 fiber.

SEQ ID NOs. 33 and 34 set forth the encoding nucleotide sequence and amino acid sequence of Ad19p fiber.

15 SEQ ID NOs. 35 and 36 set forth the encoding nucleotide sequence and amino acid sequence of Ad30 fiber.

SEQ ID NOs. 37 and 38 set forth the encoding nucleotide sequence and amino acid sequence of Ad16 fiber.

SEQ ID NOs. 39 and 40 set forth the encoding nucleotide sequence and amino acid sequence of Ad35 fiber.

20 SEQ ID NOs. 41 and 42 set forth the encoding nucleotide sequence and amino acid sequence of Ad5/Ad16 chimeric fiber. The chimeric fiber contains the N-terminal 17 amino acids from Ad5 and the remainder of the sequence is from Ad16.

25 SEQ ID NOs. 43 and 44 set forth the encoding nucleotide sequence and amino acid sequence of Ad5/Ad35 chimeric fiber. The chimeric fiber contains the N-terminal 17 amino acids from Ad5 and the remainder of the sequence is from Ad35.

-56-

1. Preparation of viral particles

The packaging cells used to produce the viruses provided herein contain the nucleic acid encoding the capsid (i.e. fiber, penton, hexon) protein. Such nucleic acid can be transfected into the cell, generally as
5 part of a plasmid, or it can be infected into the cell with a viral vector. It can be stably incorporated into the genome of the cell, thus providing for a stable cell line. Alternatively, nucleic acid encoding the heterologous or mutated capsid protein can be removed from the genome, in which case a transient complementing cell is employed.

10 The adenovirus genome to be packaged is transferred into the complementing cell by techniques known to those skilled in the art. These techniques include transfection or infection with the adenovirus. The nucleic acid encoding the mutated or heterologous fiber protein can be in this genome instead of in the packaging cell.

15 In certain cases, when the nucleic acid in the genome to be packaged encodes a mutated or heterologous fiber protein, it can be desirable for the packaging cell to also encode a fiber protein. Such protein can assist in the maturation and packaging of an infectious particle. Such protein can be a wild-type fiber protein or one modified
20 such that it is unable to attach to the penton base protein and is for use, for example, in producer cells where the fiber is included to provide the packaging function and the vector encodes a full-length fiber.

The packaging cells are cultured under conditions that permit the production of the desired viral particle. The viral particles are recovered
25 by standard techniques. An exemplary method for producing adenoviral particles provided herein is as follows. The nucleic acid encoding the mutated or heterologous capsid protein is made using standard techniques in an adenoviral shuttle plasmid. This plasmid contains the right end of the virus, in particular from the end of the E3 region through the right ITR.

-57-

This plasmid is co-transfected into competent cells of an *E. coli* strain, such as the well known *E. coli* strain BJ5183 (see, *e.g.*, Degryse (1996) *Gene* 170:45-50) along with a plasmid, which contains the remaining portion of the adenovirus genome, except for the E1 region and

5 sometimes also the E2a region and also contains a corresponding region of homology. Homologous recombination between the two plasmids generates a full-length plasmid encoding the entire adenoviral vector genome.

This full-length adenoviral vector genome plasmid is then
10 transfected into a complementing cell line. The transfection can be performed in the presence of a reagent that directs adenoviral particle entry into producer cells. Such reagents include, but are not limited to, polycations and bifunctional reagents, such as those described herein. A complementing cell, for example, is a cell of the PER.C6 cell line, which
15 contains the adenoviral E1 gene (PER.C6 is available, for example, from Crucell, The Netherlands; deposited under ECACC accession no. 96022940; see, also Fallaux *et al.* (1998) *Hum. Gene Ther.* 9:1909-1907; see, also, U.S. Patent No. 5,994,128) or an AE1-2a cell (see, Gorziglia *et al.* (1996) *J. Virology* 70:4173-4178; and Von Seggern *et al.*
20 (1998) *J. Gen. Virol.* 79:1461-1468)).

AE1-2a cells are derivatives of the A549 lung carcinoma line (ATCC # CCL 185) with chromosomal insertions of the plasmids pGRE5-2.E1 (also referred to as GRE5-E1-SV40-Hygro construct and listed in SEQ ID NO. 47) and pMNeoE2a-3.1 (also referred to as MMTV-
25 E2a-SV40-Neo construct and listed in SEQ ID NO. 48), which provide complementation of the adenoviral E1 and E2a functions, respectively.

The 633 cell line (see, von Seggern *et al.* (2000) *J. Virology* 74:354-362), which stably expresses the adenovirus serotype 5 wild-type fiber protein, and was derived from the AE1-2a cell line, is another

-58-

example of complementing cells. When the cell line is 633 cell line, the final passage of the adenoviral vector is performed on another complementing cell line (*e.g.*, Per.C6), which does not express wild-type Ad5 fiber.

- 5 The transfected complementing cells are maintained under standard cell culture conditions. The adenoviral plasmids recombine to form the adenoviral genome that is packaged. The particles are infectious, but replication deficient because their genome is missing at least the E1
10 and mutated or heterologous fiber proteins. They are recovered from the crude viral lysate, amplified, and are purified by standard techniques.

- The recovered particles can be used to infect PER.C6 or AE1-2a cells. This permits the recovery of particles whose capsids contain only the desired mutated fiber. This two-step procedure provides high titer
15 batches of the adenoviral particles provided herein. The adenoviral particles can be replication competent or replication incompetent.

- In one embodiment, the particles selectively replicate in certain predetermined target tissue but are replication incompetent in other cells and tissues. In a particular embodiment, the adenoviral particles replicate
20 in abnormally proliferating tissue, such as solid tumors and other neoplasms. In replication conditional adenoviruses, a gene essential for replication is placed under control of a heterologous promoter which is cell or tissue specific. For example, the E1a gene is placed under control of a promoter which is active in a tumor cell to produce an oncolytic
25 adenovirus or oncolytic adenoviral vector. Administration of oncolytic adenoviral vectors to tumor cells kills the tumor cells. Such replication conditional adenoviral particles and vectors can be produced by techniques known to those skilled in the art, such as those disclosed in the above-referenced U.S. Patent Nos. 5,998,205 and 5,801,029. These

-59-

particles and vectors can be produced in adenoviral packaging cells as disclosed above. Generally packaging cells are those that have been designed to limit homologous recombination that could lead to wild-type adenoviral particles. Such cells are well known and include the packaging
5 cell known as PER.C6 (see, *e.g.*, U.S. Patent Nos. 5,994,128 and 6,033,908; deposited under ECACC accession no. 96022940).

2. Adenoviral vectors and particles

The adenovirus as used herein for production of the adenoviral vectors and particles can be of any serotype, such as an Ad5 or Ad2.
10 Adenoviral stocks that can be employed as a source of adenovirus or adenoviral coat protein, such as fiber and/or penton base, can be amplified from the adenoviral serotypes 1 through 51, which are currently available from the American Type Culture Collection (ATCC, Rockville, Md.), or from any other serotype of adenovirus available from any other
15 source. For instance, an adenovirus can be of subgroup A (*e.g.*, serotypes 12, 18, 31), subgroup B (*e.g.*, serotypes 3, 7, 11, 14, 16, 21, 34, 35, 50), subgroup C (*e.g.*, serotypes 1, 2, 5, 6), subgroup D (*e.g.*, serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51), subgroup E (serotype 4), subgroup F (serotype 40, 41), or any other
20 adenoviral serotype. In certain embodiments, the adenovirus is a subgroup C adenovirus. Subgroup C adenoviruses which are modified in as described herein, include, but are not limited to, Ad2 and Ad5.

The adenoviral vectors provided herein can be used to study cell transduction and gene expression *in vitro* or in various animal models.
25 The latter case includes *ex vivo* techniques, in which cells are transduced *in vitro* and then administered to the animal. They also can be used to conduct gene therapy on humans or other animals. Such gene therapy can be *ex vivo* or *in vivo*. For *in vivo* gene therapy, the adenoviral particles in a pharmaceutically-acceptable carrier are delivered to a human

-60-

in a therapeutically effective amount in order to prevent, treat, or ameliorate a disease or other medical condition in the human through the introduction of a heterologous gene that encodes a therapeutic protein into cells in such human. The adenoviruses are delivered at a dose
5 ranging from approximately 1 particle per kilogram of body weight to approximately 10^{14} particles per kilogram of body weight. Generally, they are delivered at a dose of approximately 10^6 particles per kilogram of body weight to approximately 10^{13} particles per kilogram of body weight, and typically the dose ranges from approximately 10^8 particles per
10 kilogram of body weight to approximately 10^{12} particles per kilogram of body weight.

Any vectors known to those of skill in the art can be employed and used to produce viral particles that include fibers modified to enhance binding and infectivity of dendritic cells.

15 a. **Gutless vectors**

Gutted adenovirus vectors are those from which most or all viral genes have been deleted. They are grown by co-infection of the producing cells with a "helper" virus (such as using an E1-deleted Ad vector), where the packaging cells express the E1 gene products. The
20 helper virus trans-complements the missing Ad functions, including production of the viral structural proteins needed for particle assembly. To incorporate the capsid modifications into a gutted adenoviral vector capsid, the changes must be made to the helper virus as described herein. All the necessary Ad proteins including the modified capsid protein are
25 provided by the modified helper virus, and the gutted adenovirus particles are equipped with the particular modified capsid expressed by the host cells. The E1a, E1b, E2a, E2b and E4 are generally required for viral replication and packaging. If these genes are deleted, then the packaging cell must provide these genes or functional equivalents.

-61-

A helper adenovirus vector genome and a gutless adenoviral vector genome are delivered to packaging cells. The cells are maintained under standard cell maintenance or growth conditions, whereby the helper vector genome and the packaging cell together provide the

5 complementing proteins for the packaging of the adenoviral vector particle. Such gutless adenoviral vector particles are recovered by standard techniques. The helper vector genome can be delivered in the form of a plasmid or similar construct by standard transfection techniques, or it can be delivered through infection by a viral particle

10 containing the genome. Such viral particle is commonly called a helper virus. Similarly, the gutless adenoviral vector genome can be delivered to the cell by transfection or viral infection.

The helper virus genome can be the modified adenovirus vector genome as disclosed herein. Such genome also can be prepared or

15 designed so that it lacks the genes encoding the adenovirus E1A and E1B proteins. In addition, the genome can further lack the adenovirus genes encoding the adenovirus E3 proteins. Alternatively, the genes encoding such proteins can be present but mutated so that they do not encode functional E1A, E1B and E3 proteins. Furthermore, such vector genome

20 can not encode other functional early proteins, such as E2A, E2B3, and E4 proteins. Alternatively, the genes encoding such other early proteins can be present but mutated so that they do not encode functional proteins.

In producing the gutless vectors, the helper virus genome also is

25 packaged, thereby producing helper virus. In order to minimize the amount of helper virus produced and maximize the amount of gutless vector particles produced, the packaging sequence in the helper virus genome can be deleted or otherwise modified so that packaging of the helper virus genome is prevented or limited. Since the gutless vector

-62-

genome will have an unmodified packaging sequence, it will be preferentially packaged.

One way to do this is to mutate the packaging sequence by deleting one or more of the nucleotides comprising the sequence or otherwise mutating the sequence to inactivate or hamper the packaging function. One exemplary approach is to engineer the helper genome so that recombinase target sites flank the packaging sequence and to provide a recombinase in the packaging cell. The action of recombinase on such sites results in the removal of the packaging sequence from the helper virus genome. The recombinase can be provided by a nucleotide sequence in the packaging cell that encodes the recombinase. Such sequence can be stably integrated into the genome of the packaging cell. Various kinds of recombinase are known by those skilled in the art, and include, but are not limited to, Cre recombinase, which operates on so-called lox sites, which are engineered on either side of the packaging sequence as discussed above (see, *e.g.*, U.S. Patent Nos. 5,919, 676, 6,080,569 and 5,919,676; see, also, *e.g.*, Morsy and Caskey, Molecular Medicine Today, Jan. 1999, pgs. 18-24).

An example of a gutless vector is pAdARSVDys (Haecker *et al.* (1996) *Hum Gene Ther.* 7:1907-1914)). This plasmid contains a full-length human dystrophin cDNA driven by the RSV promoter and flanked by Ad inverted terminal repeats and packaging signals. 293 cells are infected with a first-generation Ad, which serves as a helper virus, and then transfected with purified pAdARSVDys DNA. The helper Ad genome and the pAdARSVDys DNA are replicated as Ad chromosomes, and packaged into particles using the viral proteins produced by the helper virus. Particles are isolated and the pAdARSVDys-containing particles separated from the helper by virtue of their smaller genome size and therefore different density on CsCl gradients. Other examples of

-63-

gutless adenoviral vectors are known (see, *e.g.*, Sandig *et al.* (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97(3):1002-7).

b. Oncolytic vectors

Oncolytic adenoviruses are viruses that replicate selectively in
5 tumor cells. Such vectors generally will not be useful for targeting
dendritic cells, unless such cells are malignant. Briefly, oncolytic vectors
are designed to amplify the input virus dose due to viral replication in the
tumor, leading to spread of the virus throughout the tumor mass. *In situ*
replication of adenoviruses leads to cell lysis. This *in situ* replication
10 permits relatively low, non-toxic doses to be highly effective in the
selective elimination of tumor cells. One approach to achieving selectivity
is to introduce loss-of-function mutations in viral genes that are essential
for growth in non-target cells but not in tumor cells (see, *e.g.*, U.S. Patent
No. 5,801,029). This strategy is exemplified by the use of Add11520,
15 which has a deletion in the E1b-55KD gene. In normal cells, the
adenoviral E1b-55KD protein is needed to bind to p53 to prevent
apoptosis. In p53-deficient tumor cells, E1b-55K binding to p53 is
unnecessary. Thus, deletion of E1b-55KD should restrict vector
replication to p53-deficient tumor cells.
20 Another approach is to use tumor-selective promoters to control
the expression of early viral genes required for replication (see, *e.g.*,
International PCT application Nos. WO 96/17053 and WO 99/25860).
Thus, in this approach the adenoviruses selectively replicate and lyse
tumor cells if the gene that is essential for replication is under the control
25 of a promoter or other transcriptional regulatory element that is
tumor-selective.

For example oncolytic adenoviral vectors that contain a cancer
selective regulatory region operatively linked to an adenoviral gene
essential for adenoviral replication are known (see, *e.g.*, U.S. Patent No.

-64-

5,998,205). Adenoviral genes essential for replication include, but are not limited to, E1a, E1b, E2a, E2b and E4. For example, an exemplary oncolytic adenoviral vector has a cancer selective regulatory region operatively linked to the E1a gene. In other embodiments, the oncolytic
5 adenoviral vector has a cancer selective regulatory region of the present invention operatively linked to the E1a gene and a second cancer selective regulatory region operatively linked to the E4 gene. The vectors also can include at least one therapeutic transgene, such as, but not limited to, a polynucleotide encoding a cytokine such as GM-CSF that can
10 stimulate a systemic immune response against tumor cells.

Other exemplary oncolytic adenoviral vectors include those in which expression of an adenoviral gene, which is essential for replication, is controlled by E2F-responsive promoters, which are selectively transactivated in cancer cells. Thus, vectors that contain an adenoviral
15 nucleic acid backbone that contain in sequential order: A left ITR, an adenoviral packaging signal, a termination signal sequence, an E2F responsive promoter which is operably linked to a first gene, such as E1a, essential for replication of the recombinant viral vector and a right ITR (see, published International PCT application No. WO02/06786, and U.S.
20 Patent No. 5,998,205).

In other embodiments, the oncolytic adenoviral vector has a cancer selective regulatory region operatively linked to the E1a gene and a second cancer selective regulatory region operatively linked to the E4 gene. The vectors also can carry at least one therapeutic transgene, such
25 as, but not limited to, a polynucleotide encoding a cytokine such as GM-CSF that can stimulate a systemic immune response against tumor cells.

-65-

3. Packaging

The viral particles provided herein can be made by any method known to those of skill in the art. Generally they are prepared by growing the adenovirus vector that contains nucleic acid that encodes the

5 modified or heterologous capsid protein in standard adenovirus packaging cells to produce particles that express the modified or heterologous capsid proteins. Alternatively, the vectors do not encode fiber proteins. Such vectors are packaged in producer cells to produce particles that express the modified fiber proteins.

10 As discussed, recombinant adenoviral vectors generally have at least a deletion in the first viral early gene region, referred to as E1, which includes the E1a and E1b regions. Deletion of the viral E1 region renders the recombinant adenovirus defective for replication and incapable of producing infectious viral particles in subsequently infected target cells.

15 Thus, to enable E1-deleted adenovirus genome replication and to produce virus particles requires a system of complementation which provides the missing E1 gene product. E1 complementation is typically provided by a cell line expressing E1, such as the human embryonic kidney packaging cell line, i.e. an epithelial cell line, called 293. Cell line 293 contains the

20 E1 region of adenovirus, which provides E1 gene region products to "support" the growth of E1-deleted virus in the cell line (see, *e.g.*, Graham *et al.*, *J. Gen. Virol.* 36: 59-71, 1977). Additionally, cell lines that may be usable for production of defective adenovirus having a portion of the adenovirus E4 region have been reported (WO 96/22378).

25 Multiply deficient adenoviral vectors and complementing cell lines have also been described (WO 95/34671, U.S. Patent No. 5,994,106).

For example, copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/EP00/00265, filed January 14, 200, published as International PCT application No.

-66-

WO/0042208) provides packaging cell lines that support viral vectors with deletions of major portions of the viral genome, without the need for helper viruses and also provides cell lines and helper viruses for use with helper-dependent vectors. The packaging cell line has heterologous DNA
5 stably integrated into the chromosomes of the cellular genome. The heterologous DNA sequence encodes one or more adenovirus regulatory and/or structural polypeptides that complement the genes deleted or mutated in the adenovirus vector genome to be replicated and packaged.

Packaging cell lines express, for example, one or more adenovirus
10 structural proteins, polypeptides, or fragments thereof, such as penton base, hexon, fiber, polypeptide IIIa, polypeptide V, polypeptide VI, polypeptide VII, polypeptide VIII, and biologically active fragments thereof. The expression can be constitutive or under the control of a regulatable promoter. These cell lines are particularly designed for
15 expression of recombinant adenoviruses intended for delivery of therapeutic products. For use herein, such packaging cell lines can express the modified or heterologous capsid proteins, such as the fiber proteins whose binding and infection of dendritic cells is enhanced.

Particular packaging cell lines complement viral vectors having a
20 deletion or mutation of a DNA sequence encoding an adenovirus structural protein, regulatory polypeptides E1A and E1B, and/or one or more of the following regulatory proteins or polypeptides: E2A, E2B, E3, E4, L4, or fragments thereof.

The packaging cell lines are produced by introducing each DNA
25 molecule into the cells and then into the genome via a separate complementing plasmid or plurality of DNA molecules encoding the complementing proteins can be introduced via a single complementing plasmid. Of interest herein, is a variation in which the complementing plasmid includes DNA encoding adenovirus fiber protein (or a chimeric or

-67-

modified variant thereof), from Ad virus of subgroup D, such as Ad19p or Ad37.

For applications, such as therapeutic applications, the delivery plasmid further can include a nucleotide sequence encoding a
5 heterogeneous polypeptide. Exemplary delivery plasmids include, but are not limited to, pDV44, p Δ E1B β -gal and p Δ E1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943). In a similar or analogous manner, therapeutic nucleic acids, such as nucleic acids that encode therapeutic genes, can be introduced.

10 The cell further includes a complementing plasmid encoding a fiber or other capsid protein as contemplated herein; the plasmid or portion thereof is integrated into a chromosome(s) of the cellular genome of the cell.

Typically, the packaging cell lines will contain nucleic acid encoding
15 the capsid protein or modified capsid protein stably integrated into a chromosome or chromosomes in the cellular genome. The packaging cell line can be derived from a procaryotic cell line or from a eukaryotic cell line. While various embodiments suggest the use of mammalian cells, and more particularly, epithelial cell lines, a variety of other, non-epithelial
20 cell lines are used in various embodiments. Thus, while various embodiments disclose the use of a cell line selected from among the 293, A549, W162, HeLa, Vero, 211, and 211A cell lines, any other cell lines suitable for such use are likewise contemplated herein.

25 **4. Propagation and Scale-up of doubly-ablated adenoviral vectors**

Since doubly ablated adenoviral vectors containing mutations in the fiber and/or penton capsid proteins result in inefficient cell binding and entry via the CAR/ α v integrin entry pathway, scaled up technologies improve the growth and propagation of such vectors to produce high

-68-

titers of the adenoviral vectors for clinical use. Thus, also provided is a method for scaling up the production of detargeted adenoviral vectors. The detargeted adenoviral vectors comprise an adenoviral vector modified to ablate the interaction of said vector with at least one host cell receptor compared with a wild-type adenoviral vector. The detargeted adenoviral vectors can comprise an adenoviral vector modified to ablate the interaction of said vector with one, two, three or more host cell receptors. Thus, the method is suitable for producing the detargeted adenoviral vectors disclosed herein.

10 As noted, growth and propagation of doubly and fully ablated adenoviral vectors is enhanced by new scale up technologies. Doubly ablated vectors contain mutations in the fiber and penton capsid proteins that result in inefficient cell binding and entry via the normal cellular entry pathway using CAR and integrins. These vectors are fully detargeted *in*
15 *vitro* and, thus, alternative cellular entry strategies allow for the efficient growth and generation of high titer preparations.

Two strategies have been envisioned to scale up vectors that are detargeted via fiber and/or penton modifications. These include: (a) the use of pseudoreceptor cell lines engineered to express a surface receptor
20 that binds a ligand displayed on the vector (see, *e.g.*, International PCT application No. WO 98/54346) and (b) complementing cell lines that are engineered to express native fiber and that can be engineered to express native fiber and penton (see, *e.g.*, International PCT application No. WO 00/42208). Although these systems have shown promise for scaling up
25 ablated adenoviral vectors, there is a need to develop a system for the simple, efficient production of the fully detargeted adenoviral vector for therapeutic uses.

-69-

Provided herein is a scale-up method for the propagation of detargeted adenoviral vectors. The method uses polycations and/or bifunctional reagents, which when added to tissue culture medium, bind adenoviral particles and direct their entry into the producer cells.

- 5 Reagents (also called medium additives) also can be included in the tissue culture medium containing producer cells to be infected with the detargeted adenoviral vectors. Alternatively the reagents can be pre-mixed with the virus, which mixture is then added to the tissue producer cells. The reagents can be added to tissue culture medium containing
- 10 producer cells, or producer cells can be added to tissue culture medium containing the reagents. Any suitable producer cell known to the skilled artisan can be used in the present methods. The reagents can be added at the same time that the producer cells are infected with detargeted adenoviral vectors. Generally the reagents are present in the tissue
- 15 culture medium prior to infection by the detargeted adenoviral vectors. The medium additives are maintained in the tissue culture medium during vector growth, spread and propagation. High titer yields of adenoviral vectors are obtained by this method.

- Reagents which are useful in this method are those that are
- 20 capable of directing adenoviral particle entry into the producer cells. Such reagents include, but are not limited to, polycations and bifunctional reagents. Suitable polycations include, but are not limited to, polythetylenimine; protamine sulfate; poly-L-lysine hydrobromide; poly(dimethyl diallyl ammonium) chloride (Merquat(r)-100, Merquat(r)280,
- 25 Merquat(r)550); poly-L-arginine hydrochloride; poly-L-histidine; poly(4-vinylpyridine), poly(4-vinylpyridine) hydrochloride; poly(4-vinylpyridine)cross-linked, methylchloride quaternary salt; poly(4-vinylpyridine-co-styrene); poly(4-vinylpyridinium poly(hydrogen fluoride)); poly(4-vinylpyridinium-P-toluene sulfonate); poly(4-vinylpyridinium-tribro-

-70-

mide); poly(4-vinylpyrrolidone-co-2-dimethylamino-ethyl methacrylate); polyvinylpyrrolidone, cross-linked; poly vinylpyrrolidone, poly(melamine-co-formaldehyde); partially methylated; hexadimethrine bromide; poly(Glu, Lys) 1:4 hydrobromide; poly(Lys, Ala) 3:1 hydrobromide; poly(Lys, Ala) 5 2:1 hydrobromide; poly-L-lysine succinylated; poly(Lys, Ala) 1:1 hydrobromide; and poly(Lys, Trp) 1:4 hydrobromide.

Suitable bifunctional reagents include, but are not limited to, antibodies or peptides that bind to the adenoviral capsid and that also contain a ligand that allows interaction with specific cell surface receptors of the producer cells. Examples of bifunctional reagents include: (a) 10 anti-fiber antibody ligand fusions, (b) anti-fiber-Fab-FGF conjugate, (c) anti-penton-antibody ligand fusions, (d) anti-hexon antibody ligand fusions and (e) polylysine-peptide fusions. The ligand is any ligand that will bind to any cell surface receptor found on the producer cells.

15 F. Adenovirus Expression Vector Systems

The adenovirus vector genome that is encapsulated in the virus particle and that expresses exogenous genes in a gene therapy setting is provided. The components of a recombinant adenovirus vector genome include the ability to express selected adenovirus structural genes, to 20 express a desired exogenous protein, and to contain sufficient replication and packaging signals that the genome is packaged into a gene delivery vector particle. An exemplary replication signal is an adenovirus inverted terminal repeat containing an adenovirus origin of replication, as is well known and described herein. Although adenovirus include many proteins, 25 not all adenovirus proteins are required for assembly of a recombinant adenovirus particle (vector). Thus, deletion of the appropriate genes from a recombinant Ad vector permits accommodation of even larger "foreign" DNA segments.

-71-

One recombinant adenovirus vector genome is "helper independent" so that genome can replicate and be packaged without the help of a second, complementing helper virus. Complementation is provided by a packaging cell. Particularly contemplated are helper
5 dependent systems. In an exemplary embodiment, the adenovirus vector genome does not encode a functional adenovirus fiber protein. A non-functional fiber gene refers to a deletion, mutation or other modification to the adenovirus fiber gene such that the gene does not express any or insufficient adenovirus fiber protein to package a fiber-containing
10 adenovirus particle without complementation of the fiber gene by a complementing plasmid or packaging cell line. Such a genome is referred to as a "fiberless" genome, not to be confused with a fiberless particle. Alternatively, a fiber protein may be encoded but is insufficiently expressed to result in a fiber containing particle.

15 Thus, contemplated for use are helper-independent fiberless recombinant adenovirus vector genomes that include genes that (a) express all adenovirus structural gene products but express insufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene, (b) express an exogenous
20 protein, and (c) contains an adenovirus packaging signal and inverted terminal repeats containing adenovirus origin of replication.

The adenovirus vector genome is propagated *in vitro* in the form of rDNA plasmids containing the genome, and upon introduction into an appropriate host, the viral genetic elements provide for viral genome
25 replication and packaging rather than plasmid-based propagation. Exemplary methods for preparing an Ad-vector genome are described in the Examples.

A vector herein includes a nucleic acid (such as DNA) molecule capable of autonomous replication in a cell and to which a DNA segment,

-72-

e.g., a gene or polynucleotide, can be operatively linked to bring about replication of the attached segment. For purposes herein, one of the nucleotide segments to be operatively linked to vector sequences encodes at least a portion of a therapeutic nucleic acid molecule. As noted above, therapeutic nucleic acid molecules include those encoding proteins and also those that encode regulatory factors that can lead to expression or inhibition or alteration of expression of a gene product in a dendritic cell.

1. Nucleic Acid Gene Expression Cassettes

In various embodiments, a peptide-coding sequence of the therapeutic gene is inserted into an expression vector and expressed; however, it also is feasible to construct an expression vector which also includes some non-coding sequences as well. Generally, however, non-coding sequences are excluded. Alternatively, a nucleotide sequence for a soluble form of a polypeptide may be utilized. Another therapeutic viral vector includes a nucleotide sequence encoding at least a portion of a therapeutic nucleotide sequence operatively linked to the expression vector for expression of the coding sequence in the therapeutic nucleotide sequence.

The choice of viral vector into which a therapeutic nucleic acid molecule is operatively linked depends directly, as is well known in the art, on the functional properties desired, *e.g.*, vector replication and protein expression, and the host cell to be transformed -- these being limitations inherent in the art of constructing recombinant DNA molecules. Although certain adenovirus serotypes are recited herein in the form of specific examples, it should be understood that the use of *any* adenovirus serotype, including hybrids and derivatives thereof are contemplated. Of particular interest, is the use of fiber that targets the resulting viral particle to dendritic cells.

-73-

2. Promoters

As noted elsewhere herein, an expression nucleic acid in an Ad-derived vector also include a promoter, particularly a tissue or cell specific promoter, such as one expressed dendritic cells. Promoters are nucleic acid fragments that contain a DNA sequence that controls the expression of a gene located 3' or downstream of the promoter. The promoter is the DNA sequence to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene, typically located 3' of the promoter. A promoter also includes DNA sequences which direct the initiation of transcription, including those to which RNA polymerase specifically binds. If more than one nucleic acid sequence encoding a particular polypeptide or protein is included in a therapeutic viral vector or nucleotide sequence, more than one promoter or enhancer element may be included, particularly if that would enhance efficiency of expression. Reglatable (inducible) as well as constitutive promoters may be used, either on separate vectors or on the same vector. For example, some useful regulatable promoters are those of the CREB-regulated gene family and include inhibin, gonadotropin, cytochrome c, glucagon and other. (See, *e.g.*, International PCT application No. WO 96/14061). The promoter selected can be selected from a dendritic cell-specific gene, such as NF κ B.

A regulatable or inducible promoter is a promoter where the rate of RNA polymerase binding and initiation is modulated by external stimuli. (see, *e.g.*, U.S. Patent Nos. 5,750,396 and 5,998,205). Such stimuli include various compounds or compositions, light, heat, stress, chemical energy sources, and the like. Inducible, suppressible and repressible promoters are considered regulatable promoters. Regulatable promoters also can include tissue-specific promoters. Tissue-specific promoters direct the expression of the gene to which they are operably linked to a

-74-

specific cell type. Tissue-specific promoters cause the gene located 3' of it to be expressed predominantly, if not exclusively, in the specific cells where the promoter expressed its endogenous gene. Typically, it appears that if a tissue-specific promoter expresses the gene located 3' of it at
5 all, then it is expressed appropriately in the correct cell types (see, *e.g.*, Palmiter et al. (1986) Ann. Rev. Genet. 20: 465-499).

G. Heterologous Polynucleotides and Therapeutic Nucleic Acids

The packaged adenoviral genome also can contain a heterologous polynucleotide that encodes a product of interest, such as a therapeutic
10 protein. Adenoviral genomes containing heterologous polynucleotides are well known (see, *e.g.*, U.S. Patent Nos. 5,998,205, 6,156,497, 5,935,935, and 5,801,029). These can be used for *in vitro* and *in vivo* delivery of the products of heterologous polynucleotides or the heterologous polynucleotides.

15 The adenoviral particles provided herein can be used to engineer a cell to express a protein that it otherwise does not express or does not express in sufficient quantities. This genetic engineering is accomplished by infecting the desired cell with an adenoviral particle whose genome includes a desired heterologous polynucleotide. The heterologous
20 polynucleotide is then expressed in the genetically engineered cells. For use herein the cell is generally a mammalian cell, and is typically a primate cell, including a human cell. The cell can be inside the body of the animal (*in vivo*) or outside the body (*in vitro*). Heterologous polynucleotides (also referred to as heterologous nucleic acid sequences) are included in the
25 adenoviral genome within the particle and are added to that genome by techniques known in the art. Any heterologous polynucleotide of interest can be added, such as those disclosed in U.S. Patent No. 5,998,205, incorporated herein by reference.

-75-

Polynucleotides that are introduced into an Ad genome or vector can be any that encode a protein of interest or that are regulatory sequences. In particular, the genomes can include heterologous nucleic acid encoding a product for expression in a dendritic cell for presentation or to alter the activity of the dendritic cell. For purposes herein, proteins include, but are not limited to tumor antigens. Tumor antigens included, but are not limited to carcinoembryonic antigen, NY-BR1, NY-ESO-1, MAGE-1, MAGE-3, BAGE, GAGE, SCP-1, SSX-1, SSX-2, SSX-4, CT-7, Her2/Neu, NY-BR-62, NY-BR-85 and tumor protein D52 (Scanlan and Jäger (2001) *Breast Cancer Res.* 3:95-98; Yu and Restifo (2002) *J. Clin. Invest.* 110:289-94). The following Table includes an exemplary list of tumor antigens and tissues expressing such antigens.

15

20

Antigen	Tissue
Oncofetal	
OPA	Fetal pancreas
CEA	Colon, Rectal, Stomach, Lung, Pancreas, Kidney, Bladder, Head & Neck, Cervical, endometrial, ovarian, Breast
POA	Fetal pancreas
FAP	Fetal pancreas
PA8-15	Pancreatic cancer cell line SUIT-2
Adult	
CA 50	Colorectal carcinoma cell line
CA 19-9	Colon carcinoma cell line SW1116
CA 242	Colorectal carcinoma cell line COLO 205
CAR-3	Epidermoid carcinoma cell line A 431

-76-

	Antigen	Tissue
	DU-PAN-2	Pancreatic carcinoma cell line HPAF
	Ypan-1	Pancreatic carcinoma cell line SW1990
	Span-1	"
	BW494	Pancreatic tumor tissue
5	MUSE 11	Gastric cancer ascites fluid
	L _{A1}	Embryonal carcinoma cells
	Le ^a Fuc-L _{A1}	Colon adenocarcinoma Pancreatic adenocarcinoma
	Le ^b	Colon adenocarcinoma Pancreatic adenocarcinoma
10	3-isoL _{M1}	Small cell lung carcinoma Glioma Medulloblastoma Teratocarcinoma cells
	3',6'-isoL _{D1}	Liver metastasis of colon cancer Embryonal carcinoma cells
	Fuc-3'-isoL _{M1} Sialylated Le ^a	Gastrointestinal cancer
15	Fuc-3',6'-isoL _{D1} Disialylated Le ^a	Human colon adenocarcinoma
20	nL _{A1} i-Antigen	Colon cancer Lung cancer

-77-

	Antigen	Tissue
5	SSEA-1 Le ^x Fuc-nL _{A1}	Teratocarcinoma Colon cancer
	Dimeric Le ^x	Adenocarcinoma Colon cancer Liver cancer
	Le ^y	Gastric cancer Breast cancer Colon cancer
	6'-L _{M1}	Colorectal carcinoma Lung carcinomas Primary hepatoma
10	Sialylated Le ^x or Fuc-3'-L _{M1}	Gastrointestinal cancer Lung carcinoma
		Gastric colon lung breast renal cancers
	GB3 Globo-H	Burkitt's lymphoma breast cancer
15	Sulfatide	Mucinous cystadenocarcinoma,
	Disulfated G _{A1}	Hepatocellular carcinoma
	N-Glycolylneuraminic acid	Colon cancer
	N-Glycolyl-G _{M2}	N-Glycolyl-G _{M2}
20	G _{M2} OFA-I-1 OFA-I-2	Melanoma
		Glioma
		Germ cell tumors
25	G _{D2}	Melanoma
		Neuroblastoma

-78-

	Antigen	Tissue
		Small cell lung carcinoma
		Glioma
5	G _{M3} Ag FCM1 2-39 IF43 gp-100 melanoma-associated antigen	Melanoma
10	G _{D3}	Melanoma
	HJM1	Melanoma
		Medulloblastoma
		Glioma
		Leukemia
15		Meninglioma
	9-O-Acetyl-G _{D3}	Melanoma
	Fuc-G _{M1}	Small cell lung carcinoma
	COTA	Colon, ovarian
20	SW1038 CTS	Colon prostate
	MAGE-1 MAGE-2 MAGE-3 (MZ2-E MZ2-Bb)	Lung melanocyte breast
	MUC-1	Breast pancreas
25	Lewis-Ag (GICA)	Ovarian myelin
	TAG-12	Breast ovarian
	TAG-72	colon ovarian pancreas
	Orfan-specific cancer neoantigen (OSN)	Lung
30	GP100	Melanocyte

-79-

	Antigen	Tissue
5	MART-1	Melanocyte
	p95/p97	Melanocyte
	EGF receptor	Squamous tumors
	CA125	Ovary
		Breast
10	p97 (melanotransferrin)	Melanocyte
	22-1-1	uterus cervix ovary
	GA733	gastrointestinal carcinoma
	YH206	adenocarcinomas
	MART-2	melanocytes
15	BAGE-1	melanocytes
	GAGE1-6	melaocyte
		osteocarcoma
	DF3	Breast
		lymphocytes
20	L3p40-50 L3p90	Lung
	Thomsen-Friedenrich Pan Tumor Antigen	pancarcinoma
		pancreas
		ovarian
	EPB-2	B cell lymphoma
25		melanoma
		lymphoma
		medullary thyroid carcinoma
		gastrointestinal carcinoma

-80-

Antigen	Tissue
NS-ESO-1	melanoma, breast, bladder, prostate, hepatocellular carcinoma
NY-ESO-1	melanoma, breast, bladder, prostate, hepatocellular carcinoma

Proteins also include, but are not limited to, therapeutic proteins, such as an immunostimulating protein, such as an interleukin, interferon, or colony stimulating factor, such as granulocyte macrophage colony stimulating factor (GM-CSF; see, *e.g.*, 5,908,763F. Generally, such GM-CSF is a primate GM-CSF, including human GM-CSF. Other immunostimulatory genes include, but are not limited to, genes that encode cytokines IL-1, IL-2, IL-4, IL-5, IFN, TNF, IL-12, IL-18, and flt3, proteins that stimulate interactions with immune cells (B7, CD28, MHC class I, MHC class II, TAPs), tumor-associated antigens (immunogenic sequences from MART-1, gp100 (pmel-17), tyrosinase, tyrosinase-related protein 1, tyrosinase-related protein 2, melanocyte-stimulating hormone receptor, MAGE1, MAGE2, MAGE3, MAGE12, BAGE, GAGE, NY-ESO-1, -catenin, MUM-1, CDK-4, caspase 8, KIA 0205, HLA-A2R1701, -fetoprotein, telomerase catalytic protein, G-250, MUC-1, carcinoembryonic protein, p53, Her2/neu, triosephosphate isomerase, CDC-27, LDLR-FUT, telomerase reverse transcriptase, and PSMA), cDNAs of antibodies that block inhibitory signals (CTLA4 blockade), chemokines (MIP1, MIP3, CCR7 ligand, and calreticulin), and other proteins.

Other polynucleotides, including therapeutic nucleic acids, such as therapeutic genes, of interest include, but are not limited to, anti-angiogenic, and suicide genes. Anti-angiogenic genes include, but are not limited to, genes that encode METH-1, METH -2, TrpRS fragments, proliferin-related protein, prolactin fragment, PEDF, vasostatin, various fragments of extracellular matrix proteins and growth factor/cytokine

-81-

inhibitors. Various fragments of extracellular matrix proteins include, but are not limited to, angiostatin, endostatin, kininostatin, fibrinogen-E fragment, thrombospondin, tumstatin, canstatin, and restin. Growth factor/cytokine inhibitors include, but are not limited to, VEGF/VEGFR antagonist, sFlt-1, sFlk, sNRP1, angiopoietin/tie antagonist, sTie-2, chemokines (IP-10, PF-4, Gro-beta, IFN-gamma (Mig), IFN, FGF/FGFR antagonist (sFGFR), Ephrin/Eph antagonist (sEphB4 and sephrinB2), PDGF, TGF and IGF-1.

A "suicide gene" encodes a protein that can lead to cell death, as with expression of diphtheria toxin A, or the expression of the protein can render cells selectively sensitive to certain drugs, *e.g.*, expression of the Herpes simplex thymidine kinase gene (HSV-TK) renders cells sensitive to antiviral compounds, such as acyclovir, gancyclovir and FIAU (1-(2-deoxy-2-fluoro- β -D-arabinofuranosil)-5-iodouracil). Other suicide genes include, but are not limited to, genes that encode carboxypeptidase G2 (CPG2), carboxylesterase (CA), cytosine deaminase (CD), cytochrome P450 (cyt-450), deoxycytidine kinase (dCK), nitroreductase (NR), purine nucleoside phosphorylase (PNP), thymidine phosphorylase (TP), varicella zoster virus thymidine kinase (VZV-TK), and xanthine-guanine phosphoribosyl transferase (XGPRT). Alternatively, a therapeutic nucleic acid can exert its effect at the level of RNA, for instance, by encoding an antisense message or ribozyme, a protein that affects splicing or 3' processing (*e.g.*, polyadenylation), or a protein that affects the level of expression of another gene within the cell, *e.g.* by mediating an altered rate of mRNA accumulation, an alteration of mRNA transport, and/or a change in post-transcriptional regulation. The addition of a therapeutic nucleic acid to a virus results in a virus with an additional antitumor mechanism of action. Thus, a single entity (*i.e.*, the virus carrying a therapeutic transgene) is capable of inducing multiple antitumor

-82-

mechanisms. Other encoded proteins, include, but are not limited to, herpes simplex virus thymidine kinase (HSV-TK), which is useful as a safety switch (see, U.S. Patent Application No. 08/974,391, filed November 19, 1997, which published as PCT Publication No.

5 WO/9925860), Nos, FasL, and sFasR (soluble Fas receptor).

Also contemplated are combinations of two or more transgenes with synergistic, complementary and/or nonoverlapping toxicities and methods of action. The resulting adenovirus can retain the viral oncolytic functions and, for example, additionally are endowed with the ability to
10 induce immune and anti-angiogenic responses and other responses as desired.

Therapeutic polynucleotides and heterologous polynucleotides also include those that exert an effect at the level of RNA or protein. These include a factor capable of initiating apoptosis, RNA, such as RNAi and
15 other double-stranded RNA, antisense and ribozymes, which among other capabilities can be directed to mRNAs encoding proteins essential for proliferation, such as structural proteins, transcription factors, polymerases, genes encoding cytotoxic proteins, genes that encode an engineered cytoplasmic variant of a nuclease (*e.g.* RNase A) or protease
20 (*e.g.* trypsin, papain, proteinase K and carboxypeptidase). Other polynucleotides include a cell or tissue specific promoters, such as those used in oncolytic adenoviruses (see, *e.g.*, U.S. Patent No. 5,998,205).

The heterologous polynucleotide encoding a polypeptide also can contain a promoter operably linked to the coding region. Generally the
25 promoter is a regulated promoter and transcription factor expression system, such as the published tetracycline-regulated systems, or other regulatable systems (WO 01/30843), to allow regulated expression of the encoded polypeptide. Exemplary of other promoters, are tissue-selective promoters, such as those described in U.S. Patent No. 5,998,205. An

-83-

exemplary regulatable promoter system is the Tet-On (and Tet-Off) system currently available from Clontech (Palo Alto, CA). This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. This system
5 can be used to control the expression of the encoded polypeptide in the viral particles and nucleic acids provided herein. Other regulatable promoter systems are known (see, *e.g.*, published U.S. Application No. 20020168714, entitled "Regulation of Gene Expression Using Single-Chain, Monomeric, Ligand Dependent Polypeptide Switches,"
10 which describes gene switches that contain ligand binding domains and transcriptional regulating domains, such as those from hormone receptors). Other suitable promoters that can be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter and/or the E3 promoter; or heterologous promoters, such as the
15 cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; and the ApoA1 promoter.

Therapeutic transgenes can be included in the viral constructs and
20 resulting particles. Among these are those that result in an "armed" virus. For example, rather than delete E3 region as in some embodiments described herein, all or a part of the E3 region can be preserved or re-inserted in an oncolytic adenoviral vector (discussed above). The presence of all or a part of the E3 region can decrease the
25 immunogenicity of the adenoviral vector. It also increases cytopathic effect in tumor cells and decreases toxicity to normal cells. Typically such vector expresses more than half of the E3 proteins.

Adenoviruses for therapy, including those for human therapy, are known. Such known viruses can be modified as provided herein to

-84-

increase infection of dendritic cells and/or increasing binding to receptors expressed on dendritic cells. The adenoviral vectors that are used to produce the viral particles can include other modifications. Modifications include modifications to the adenovirus genome that is packaged in the

5 particle in order to make an adenoviral vector. As discussed above, adenovirus vectors and particles with a variety of modifications are available. Modifications to adenoviral vectors include deletions known in the art, such as deletions in one or more of the E1, E2a, E2b, E3, or E4 coding regions. These adenoviruses are sometimes referred to as early

10 generation adenoviruses and include those with deletions of all of the coding regions of the adenoviral genome ("gutless" adenoviruses, discussed above) and also include replication-conditional adenoviruses, which are viruses that replicate in certain types of cells or tissues but not in other types as a result of placing adenoviral genes essential for

15 replication under control of a heterologous promoter (discussed above; see also U.S. Patent No. 5,998,205, U.S. Patent No. 5,801,029; U.S. patent application 60/348,670 and corresponding published International PCT application No. WO 02/06786). These include the cytolytic, cytopathic viruses (or vectors), including the oncolytic viruses discussed

20 above.

Alternatively, as discussed above, the vector can include a mutation or deletion in the E1b gene. Typically such mutation or deletion in the E1b gene is such that the E1b-19kD protein becomes non-functional. This modification of the E1b region can be combined with

25 vectors where all or a part of the E3 region is present.

H. Formulation and administration

1. Formulation

Compositions containing therapeutically effective concentrations of recombinant adenovirus delivery vectors for delivery of therapeutic

-85-

gene products to target cells and/or tissues (i.e. dendritic cells). Modes of administration include, but are not limited to, intramuscular, parenteral, local, topical and other routes whereby dendritic cells can be targeted.

The recombinant viral compositions also can be formulated for in
5 sustained released formulations, such as adsorbed to biodegradable supports, including collagen sponges, or in liposomes. Sustained release formulations can be formulated for multiple dosage administration, so that during a selected period of time, such as a month or up to about a year, several dosages are administered. Thus, for example, liposomes can be
10 prepared such that a total of about two to up to about five or more times the single dosage is administered in one injection. The vectors are formulated in pharmaceutically acceptable carriers.

The composition can be provided in a sealed sterile vial containing an amount such that upon administration a sufficient amount of viral
15 particles is delivered where about 50 to 150 μ l, containing at least about 10^7 , or 10^8 plaque forming units (pfu) in such volume are delivered and at least 10^9 - 10^{10} pfu are delivered.

To prepare compositions the viral particles are dialyzed into a suitable carrier or viral particles can be concentration and/or mixed
20 therewith. The resulting mixture can be a solution, suspension or emulsion. In addition, the viral particles may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active agents for the particular disorder treated.

Exemplary suitable carriers include, but are not limited to,
25 physiological saline, phosphate buffered saline (PBS), balanced salt solution (BSS), lactate Ringers solution, and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions also can be suitable as pharmaceutically acceptable carriers. These can

-86-

be prepared according to methods known to those skilled in the art.

The compositions can be prepared with carriers that protect them against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and other types of implants that may be placed directly into the body. The compositions also can be administered in pellets, such as Elvax pellets (ethylene-vinyl acetate copolymer resin).

Liposomal suspensions, including tissue-targeted liposomes, also can be suitable as pharmaceutically acceptable carriers. For example, liposome formulations may be prepared by methods known to those of skill in the art (see, *e.g.*, Kimm *et al.* (1983) *Bioch. Bioph. Acta* 728:339-398; Assil *et al.* (1987) *Arch Ophthalmol.* 105:400; and U.S. Patent No. 4,522,811). The viral particles can be encapsulated into the aqueous phase of liposome systems.

The active materials also can be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action or have other action, including viscoelastic materials, such as hyaluronic acid, which is sold under the trademark HEALON, which is a solution of a high molecular weight (MW) of about 3 millions fraction of sodium hyaluronate (manufactured by Pharmacia, Inc; see, *e.g.*, U.S. Patent Nos. 5,292,362, 5,282,851, 5,273,056, 5,229,127, 4,517,295 and 4,328,803). Additional active agents may be included.

The compositions can be enclosed in ampules, disposable syringes or multiple or single dose vials made of glass, plastic or other suitable material. Such enclosed compositions can be provided in kits. In particular, kits containing vials, ampules or other container.

-87-

Finally, the vectors can be packaged as articles of manufacture containing packaging material, typically a vial, a pharmaceutically acceptable composition containing the viral particles and a label that indicates the therapeutic use of the composition.

- 5 Also provided are kits for practice of the methods herein. The kits contain one or more containers, such as sealed vials, with sufficient composition for single dosage administration, other reagents as needed, and optionally instructions for use.

- Administration of the composition is typically by intravenous or
10 intramuscular injection, although other modes of administration can be effective.

2. Administration

- The compositions containing the compounds are generally administered systemically. It is further understood that, for any particular
15 subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the recombinant viruses, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the methods,
20 uses, and products provided herein.

- In addition to *in vivo* administration, the viral particles provided herein can be used in methods of *ex vivo* therapy in which mixtures of cells, such as bone marrow cells, that include or contain dendritic cells or that are enriched for dendritic cells are contacted with the viral particles
25 so that dendritic cells are preferentially infected. The resulting cells are optionally culture *in vitro* and are then infused into a recipient subject, generally the donor.

-88-

I. Diseases, Disorders and therapeutic products

Dendritic cells modified with adenoviral particles provided herein express heterologous proteins that can be presented or that can alter dendritic cell functioning. As noted, the adenoviral particles can be administered to a subject or can be contacted *ex vivo* with dendritic cells obtained from a donor and can be infused into a subject patient, typically the donor. The viral particles, which express fibers targeted to dendritic cells will preferentially infect dendritic cells. Dendritic cells modified to express particular antigens act as vaccines by stimulating an immune response against the presented antigen. These cells can be used for treatment or prophylaxis of virtually any bacterial, protozoan, parasitic, fungal or other infection. In addition, presentation of a tumor antigen renders such cells effective for treatment or prophylaxis of cancers.

Expression of a product that interferes with dendritic cell function, such as by blocking expression of genes, including genes encoding NFκB or RelB, prevent the dendritic cells from stimulating T-cells. Such particles and the resulting cells can be used to treat diseases such as asthma, allergies, autoimmune diseases, such as juvenile diabetes, rheumatoid arthritis, lupus and inflammatory diseases.

Pathogens include, but are not limited to, bacterial, such as *E. coli* and anthrax, viruses, such as vaccinia virus (*i.e.* small pox, chicken pox), herpes viruses, cytomegalovirus (CMV) vectors, papillomavirus, parasites and fungi. Selected antigens can be determined empirically by identifying those that are effective in generating an immunoprotective response in a model system such as a rodent model.

Treatment with the particles, either *in vivo* or *ex vivo* can be prophylactic where administration (vaccination) generates immunity or it can be for treatment of the disease.

-89-

J. Examples

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1**5 Construction of Ad5 Vectors Containing the Fiber AB Loop, KO1 and Penton, PD1 Mutations and Derivatives Thereof**

Three recombinant adenoviral vectors were prepared that contain the KO1 fiber or PD1 penton base mutations either alone or in combination, these vectors are designated Av3nBgFKO1 Av1nBgPD1, and
10 Av1nBgFKO1PD1. Construction of these vectors is described below and a general description of each vector is set forth in Table 1.

TABLE 1
 Description Of Detargeted
 Recombinant Adenoviral Vectors Used For Scale-up
 Vector

15

Vector	Description
Av3nBg	An E1, E2a, E3-deleted adenoviral vector encoding a nuclear localizing β -galactosidase
Av1nBg	An E1 and E3-deleted adenoviral vector encoding a nuclear localizing β -galactosidase
Av3nBgFKO1	The same as Av3nBg but containing the KO1 mutation in the fiber gene
20 Av1nBgPD1	The same as Av1nBg but containing the PD1 mutation in the penton gene
Av1nBgFKO1PD1	The same as Av1nBg but containing the fiber KO1 and penton PD1 mutations

Av1nBg

This is a well-known vector, its sequence is set forth in SEQ ID
25 NO. 51.

Av3nBg

This is a well-known vector, its sequence is set forth in SEQ ID
 NO. 52.

-90-

Av3nBgFKO1

Genetic incorporation of the KO1 fiber mutation to generate Av3nBgFKO1

The adenoviral vector Av3nBgFKO1 was generated in an E1-, E2a-,
5 E3-deleted backbone based on the adenovirus serotype 5 genome. It contains a RSV promoted nuclear-localizing β -galactosidase gene in place of the E1 region. In addition, the fiber gene carries the KO1 mutation. This mutation results in a substitution of fiber amino acids 408 and 409, changing them from serine and proline to glutamic acid and alanine,
10 respectively.

The vector was constructed as follows. First, the plasmid pSKO1 (Figure 1) was digested with the restriction enzymes SphI and MunI. The resulting DNA fragments were separated by electrophoresis on an agarose gel. The 1601 bp fragment containing all but the 5' end of the fiber gene
15 was excised from the agarose gel and the DNA was isolated and purified. The fragment was then ligated with the 9236 bp fragment of p5FloxHRFRGD, which had been digested with SphI and MunI. The resulting plasmid, p5FloxHRFKO1, was digested with SpeI and PacI and the 6867 bp fragment containing the fiber gene was isolated. The
20 fragment was ligated with the 24,630 bp SpeI-PacI fragment of pNDSQ3.1. The resulting plasmid, pNDSQ3.1KO1 (Figure 2), was used together with pAdmireRSVnBg (Figure 3A) to generate a plasmid which encodes the full-length adenoviral vector genome. It, however, was necessary to remove the PacI site from pNDSQ3.1KO1 (Figure 2) prior to
25 recombination with pAdmireRSVnBg (Figure 3A) so that the final plasmid contains a unique PacI site adjacent to the 5' ITR. The PacI site in pNDSQ3.1KO1 was removed by digestion with PacI followed by blunting with T4 DNA Polymerase and religation. The resulting plasmid was called pNDSQ3.1KO1(Pac.

-91-

To generate a full-length plasmid containing the entire adenoviral genome, pAdmireRSVnBg (Figure 3A) was digested with Sall and co-transfected into competent cells of the *E. coli* strain BJ5183 along with pNDSQ3.1KO1ΔPac, which had been digested with BstBI.

- 5 Homologous recombination between the two plasmids generated a full-length plasmid encoding the entire adenoviral vector genome, which was called pFLAv3nBgFKO1.

- The plasmid pFLAv3nBgKO1 was linearized with PacI and transfected into 633 cells. In the fiber complementing 633 cell line, the
10 resulting viral DNA containing the KO1 mutation is capable of being packaged into infectious viral particles containing a mixture of wildtype fiber and mutant fiber proteins. After five rounds of amplification in 633 cells, a cytopathic effect was observed. Three more rounds of amplification in 633 cells were performed followed by purification of the
15 virus by standard CsCl centrifugation procedures. This viral preparation was used to infect AE1-2a cells, which do not express fiber. The resulting virus contained only the mutant fiber protein on its capsid. Virus particles were purified by standard CsCl centrifugation procedures.

Av1nBgFKO1

- 20 The vector Av1nBgFKO1 is made in a similar manner to Av3nBgFKO1 described above.

Av1nBgKO12

- An additional fiber AB loop mutation (described by Einfeld *et al.* (2001) *J. Virology* 75:11284-11291) was incorporated into the genome
25 of Av1nBg. This AB loop mutation is a four amino acid substitution, R512S, A515G, E516G, and K517G, and is referred to as KO12. The KO12 mutation was incorporated into the fiber gene by PCR gene overlap extension using the plasmid pSQ1 (Figure 3B) as template. The pSQ1 plasmid contains most of the Ad5 genome, extending from base pair

-92-

3329 through the right ITR, in a pBR322 backbone. First, a segment of the Ad5 genome extending from within the E3 region into the fiber gene was amplified by PCR using the plasmid pSQ1 as a template with the following primers termed 5FF, 5'-GAA CAG GAG GTG AGC TTA GA-3' (SEQ ID NO. 53), and 5FR, 5'-TCC GCC TCC ATT TAG TGA ACA GTT AGG AGA TGG AGC TGG TGT G-3' (SEQ ID NO. 54). The primer 5FR contains an 18 base 5'-extension that encodes the modified fiber AB loop amino acids from 512 through 517. A second PCR using pSQ1 as a template amplified the region immediately 3' of the AB loop substitution and extending past the MunI site located 40 base pairs 3' of the fiber gene stop codon. The two primers used for this reaction were 3FF: 5'-TCA CTA AAT GGA GGC GGA GAT GCT AAA CTC ACT TTG GTC TTA AC-3' (SEQ ID NO. 55), and 3FR: 5'-GTG GCA GGT TGA ATA CTA GG-3' (SEQ ID NO. 56). The primer 3FR contains an 18 base 5'-extension that encodes the modified fiber AB loop amino acids 512 through 517. Amplified products of the expected size were obtained and used in a second PCR with the end primers 5FF and 3FR to join the fragments together. The KO12 PCR fragment was digested with XbaI and MunI cloned directly into the fiber shuttle plasmid, pFBshuttle(EcoRI) to generate the plasmid pFBSEKO12 which contains the 8.8kB EcoRI fragment of pSQ1. The pFBSEKO12 plasmid was digested with XbaI and EcoRI and cloned into pSQ1 using a three-way ligation to generate pSQ1KO12 (Figure 3C). The KO12 cDNA was incorporated into the genome of Av1nBg, an adenovirus vector with E1 and E3 deleted encoding β -galactosidase, by homologous recombination between ClaI-linearized pSQ1KO12 and pAdmireRSVnBg digested with Sall and PacI to generate Av1nBgKO12. The KO12 vector was transfected in 633 cells, scaled-up on non-fiber expressing cells and purified, as described above for KO1.

-93-

Av1nBgPD1**Genetic incorporation of the PD1 penton mutation to generate Av1nBgPD1**

The adenoviral vector Av1nBgPD1 is an E1-, E3-deleted vector
5 based on the adenovirus serotype 5 genome. It contains a RSV promoted nuclear-localizing β -galactosidase gene in the E1 region and also contains the PD1 mutation in the penton gene. The PD1 mutation results in a substitution of amino acids 337 through 344 of the penton protein, HAIRGDTF (SEQ ID NO. 49), with amino acids SRGYPYDVPDYAGTS
10 (SEQ ID NO. 50), thus replacing the RGD tripeptide (see, Einfeld *et al.* (2001) *J. Virology* 75:11284-11291). The mutation in the penton gene was generated in the plasmid pGEMpen5, which contains the Adenovirus serotype 5 penton gene. To generate the mutation, four oligonucleotides were synthesized. The sequences of the oligonucleotides were as
15 follows: penton 1: 5' CGC GGA AGA GAA CTC CAA CGC GGC AGC CGC GGC AAT GCA GCC GGT GGA GGA CAT GAA 3' (SEQ ID NO. 57); penton 2: 5' TAT CGT TCA TGT CCT CCA CCG GCT GCA TTG CCG CGG CTG CCG CGT TGG AGT TCT CTT CC 3' (SEQ ID NO. 58); penton 3: 5' CGA TAG CCG CGG CTA CCC CTA CGA CGT GCC CGA CTA CGC
20 GGG CAC CAG CGC CAC ACG GGC TGA GGA GAA GCG CGC 3' (SEQ ID NO. 59); penton 4: 5' TCA GCG CGC TTC TCC TCA GCC CGT GTG GCG CTG GTG CCC GCG TAG TCG GGC ACG TCG TAG GGG TAG CCG CGG C 3' (SEQ ID NO. 60). The complementary oligonucleotides penton 1 and penton 2 were annealed to each other as were penton 3 and
25 penton 4. The duplex generated by annealing penton 3 and penton 4 encoded the substitution of amino acids 337 through 344 described above. The duplex generated by annealing penton 1 and penton 2 possessed a 5 base 5' overhang which was compatible to a 5 base 5' overhang on the duplex generated by annealing penton 3 and penton 4.

-94-

The opposite end of the duplex generated by annealing penton 1 and penton 2 contained an Earl compatible overhang. The opposite end of the duplex generated by annealing penton 3 and penton 4 contained a BbvCI compatible overhang. The two duplexes were ligated to each other and
5 ligated back into the pGEMpen5 backbone as follows. First, pGEMpen5 was digested with BbvCI and PstI and the resulting DNA fragments were separated by electrophoresis on an agarose gel. The 3360 bp fragment was excised from the gel and purified. The plasmid pGEMpen5 was also digested with PstI and Earl and the resulting fragments were separated by
10 electrophoresis on an agarose gel. The 955 bp fragment was excised from the gel and purified. These two fragments from the pGEMpen5 plasmid were ligated with the two pairs of annealed oligonucleotides to generate the plasmid pGEMpen5PD1.

The mutated penton gene was transferred from pGEMpen5PD1 to
15 pSQ1 using a 5-way ligation as follows. First, the region of the penton gene containing the PD1 mutation was excised from pGEMpen5PD1 by digestion with PvuI and Ascl. The 974 bp fragment containing the PD1 mutation was purified. Four DNA fragments were prepared from the pSQ1 plasmid (Figure 3B) as follows. The plasmid was digested with
20 Csp45I and FseI and the 9465 bp fragment was purified. In addition pSQ1 was digested with FseI and PvuI and the 2126 bp fragment was purified. The plasmid pSQ1 was digested with Ascl and BamHI and the 5891 bp fragment was purified. Finally, pSQ1 was digested with BamHI and Csp45I and the 14610 bp fragment was purified. The 5 purified DNA
25 fragments were ligated to each other to form the plasmid pSQ1PD1 (Figure 4).

To generate adenoviral vector, pSQ1PD1 was linearized by digestion with ClaI and co-transfected into PerC6 cells with pAdmireRSVnBg (Figure 3A) which had been digested with Sall and PacI.

-95-

hexadimethrine bromide was maintained in the medium at 4 μ g/ml. When a cytopathic effect was observed, a crude viral lysate was further expanded on PerC6 cells. The virus was purified by standard CsCl centrifugation procedures.

5 **Av1nBgFKO1PD1**

Genetic incorporation of the fiber KO1 or KO12 mutation in combination with the penton PD1 mutation to generate Av1nBgFKO1PD1

The adenoviral vectors Av1nBgFKO1PD1 and Av1nBgKO12PD1
10 were generated in an E1-, E3-deleted adenovirus serotype 5 genome. Both vectors contains a RSV promoted nuclear-localizing β -galactosidase gene in the E1 region and also contains either the KO1 or KO12 mutation in the fiber gene as well as the PD1 mutation in the penton gene. The vectors were constructed as follows. First, the plasmid pSQ1PD1 was
15 digested with Csp45I and SpeI and the 23976 bp fragment containing the PD1 mutated penton gene was purified. In addition, the plasmids pSQ1KO1 or pSQ1KO12 (Figure 3B) were digested with Csp45I and SpeI and the 9090 bp fragment containing the KO1 or KO12 mutated fiber gene were purified. The appropriate purified fragments were ligated to
20 each other to form the plasmid pSQ1FKO1PD1 (Figure 5A) or pSQ1KO12PD1 (Figure 5B) that contains the KO1 (or KO12) mutated fiber gene and the PD1 mutated penton gene. To generate virus, pSQ1FKO1PD1 or pSQKO12PD1 was linearized with ClaI and co-transfected into 633 cells with pAdmireRSVnBg (Figure 3A) which had been
25 digested with Sall and PacI. After three rounds of amplification in 633 cells a cytopathic effect was observed and the crude viral lysate was then amplified on PerC6 cells. Hexadimethrine bromide was maintained in the medium at 4 μ g/ml. Each virus was purified by standard CsCl centrifugation procedures.

-96-

EXAMPLE 2***In Vitro* Evaluation of Adenoviral Vectors Containing the KO1 and PD1 Mutations**

Several recombinant adenoviral vectors were used in these studies
5 to demonstrate the function of the KO1 fiber mutation and included
Av1nBg, Av1nBgFKO1, Av1nBgPD1, and Av1nBgFKO1PD1, described
above. The transduction efficiencies of adenoviral vectors containing the
KO1 and/or PD1 mutations were evaluated on cells of the alveolar
epithelial cell line A549. The transduction efficiencies were compared to
10 that of Av1nBg, an adenoviral vector containing wild type fiber and
penton.

The day prior to infection, cells were seeded into 24-well plates at
a density of approximately 1×10^5 cells per well. Immediately prior to
infection, the exact number of cells per well was determined by counting
15 a representative well of cells. Each of the vectors, Av1nBg,
Av1nBgFKO1, and Av1nBgFKO1PD1 were used to transduce A549 cells
at each of the following particle per cell (PPC) ratios: 100, 500, 1000,
2500, 5000, 10,000. The cell monolayers were stained with X-gal 24
hours after infection and the percentage of cells expressing
20 β -galactosidase was determined by microscopic observation and counting
of cells. Transductions were done in triplicate and three random fields in
each well were counted, for a total of nine fields per vector.

The results at the 500 PPC ratio are shown in Figure 6 and show a
significantly reduced transduction efficiency on A549 cells using vectors
25 containing the KO1 mutation alone or when combined with PD1
compared to Av1nBg. The vectors containing the PD1 mutation alone
had no effect on adenoviral transduction of A549 cells *in vitro*.

-97-

EXAMPLE 3

***In Vivo* Analysis of Adenoviral Vectors Containing the FK01 and PD1 Mutations**

- This Example provides experiments that evaluate the *in vivo* biodistribution of adenoviral vectors containing the KO1 and PD1 mutations and their influence on adenoviral-mediated liver transduction. The results show that ablating the viral interaction with CAR and/or integrins is not sufficient to fully detarget adenoviral vectors from the liver *in vivo*.
- 5 A positive control cohort received Av1nBg and a negative control group received HBSS. Additionally, the Av1nBgFKO12 and Av1nBgFKO12PD1 vectors were analyzed *in vivo*. These vectors each contain a fiber protein with the four amino acid substitution in the AB loop. Additionally, Av1nBgFKO12PD1 contains a mutation in the penton
- 10 base. Both of these mutations were known (see, Einfeld *et al.* (2001) *J. Virology* 75:11284-11291), and were alleged to decrease liver transduction 10 to 700 fold, respectively. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector
- 15 administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine
- 20 vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay.
- 25 For β -galactosidase immunohistochemistry slices of liver, approximately 2-3 mm thick, were placed in 10% neutral buffered formalin. After fixation, these samples were embedded in paraffin,

-98-

sectioned, and analyzed by immunohistochemistry for β -galactosidase expression. A 1:1200 dilution was used of a rabbit anti- β -galactosidase antibody (ICN Pharmaceuticals, Inc.; Costa Mesa, CA) in conjunction with a Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA) to

5 visualize positive cells.

The chemiluminescent β -galactosidase activity assay was performed using the Galacto-Light Plus™ chemiluminescent assay (Tropix, Inc., Foster City, CA) system. Tissue samples were collected in lysis matrix tubes containing two ceramic spheres (Bio101, Carlsbad, CA) and frozen on dry ice. The tissues were thawed and 500 μ l of lysis buffer from the Galacto-Light Plus kit was added to each tube. The tissue was homogenized for 30 seconds using a FastPrep System (Bio101, Carlsbad, CA). Liver samples were homogenized for an additional 30 seconds. β -galactosidase activity was determined in the liver homogenates

15 according to the manufacture's protocol.

For hexon PCR analysis DNA from tissues was isolated using the Qiagen Blood and Cell Culture DNA Midi or Mini Kits (Qiagen Inc., Chatsworth, CA). Frozen tissues were partially thawed and minced using sterile disposable scalpels. Tissues were then lysed by incubation overnight at 55° C in Qiagen buffer G2 containing 0.2 mg/ml RNaseA and 0.1 mg/ml protease. Lysates were vortexed briefly and then applied to Qiagen-tip 100 or Qiagen-tip 25 columns. Columns were washed and DNAs were eluted as described in the manufacturer's instructions. After precipitation, DNAs were dissolved in water and the concentrations were spectrophotometrically determined (A260 and A280) on a DU-600 (Beckman Coulter, Inc.; Fullerton, CA) or a SPECTRAmax PLUS (Molecular Devices, Inc.; Sunnyvale, CA) spectrophotometer. 2.3.2.

-99-

PCR primers and a Taqman probe specific to adenovirus hexon sequences were designed using Primer Express software v. 1.0 (Applied Biosystems, Foster City, CA). Primer and probe sequences were:

Hexon Forward Primer (SEQ ID NO. 61):

5'-CTTCGATGATGCCGCAGTG-3'

Hexon Reverse Primer (SEQ ID NO. 62):

5'-GGGCTCAGGTACTCCGAGG-3'

Hexon Probe (SEQ ID NO. 63):

5'-FAM-TTACATGCACATCTCGGGCCAGGAC-TAMRA-3'

Amplification was performed in a reaction volume of 50 μ l under the following conditions: 10 ng (tumor) or 1 μ g (liver and lung) of sample DNA, 1X Taqman Universal PCR Master Mix (Applied Biosystems), 600 nM forward primer, 900 nM reverse primer and 100 nM hexon probe. Thermal cycling conditions were: 2 minute incubation at 50° C, 10 minutes at 95° C, followed by 35 cycles of successive incubation at 95° C for 15 seconds and 60° C for 1 minute. Data was collected and analyzed using the 7700 Sequence Detection System software v. 1.6.3 (Applied Biosystems). Quantification of adenovirus copy number was performed using a standard curve that includes dilutions of adenovirus DNA from 1,500,000 copies to 15 copies in the appropriate background of cellular genomic DNA. For analysis of tumor tissues, a standard curve in a background of 10 ng human DNA was generated. For analysis of mouse liver and lung tissues, a standard curve using the same adenovirus DNA dilutions in a background of 1 μ g CD-1 mouse genomic DNA was generated. Samples were amplified in triplicate, and the average number of total copies was normalized to copies per cell based on the input DNA weight amount and a genome size of 6×10^9 bp.

The results of the β -galactosidase activity assay and adenoviral hexon DNA content for liver transduction by these vectors are shown in

-100-

Figure 7A and 7B. The vector containing the KO1 or KO12 mutations alone showed, on average, a slight increase in liver transduction compared to Av1nBg, which is consistent with several previous experiments. The vectors containing the PD1 mutation alone or combined
5 with KO1 or KO12 showed a slight decrease in liver transduction compared to Av1nBg, suggesting that integrins are involved to some extent in hepatic uptake of the adenoviral vectors.

The results of the immunohistochemical staining of liver sections for β -galactosidase were consistent with the activity assays (data not
10 shown) and demonstrate that gene expression was localized specifically to hepatocytes. The vectors containing the KO1 or KO12 mutation alone showed a slight increase in liver transduction as revealed by a more intense and frequent immunohistochemical-staining pattern. The vectors containing the PD1 mutation, either alone or combined with KO1 or
15 KO12, showed little difference in transduction compared to Av1nBg. These results demonstrate that ablating the viral interaction with CAR and/or integrins is not sufficient to fully detarget adenoviral vectors from the liver *in vivo*.

In summary, the fiber AB loop mutation contained in Av1nBgFKO1
20 or Av1nBgKO12 ablates interaction with human and mouse CAR *in vitro* and diminished transduction *in vitro*. *In vivo*, however, fiber AB loop mutations behaved unexpectedly, because such mutations were found to enhance adenoviral-mediated gene transfer to liver and results in increasing vector potency. The penton base, PD1 mutation that ablates
25 interaction with the second receptor involved in adenoviral internalization had no effect *in vitro* and little to no effect *in vivo*. These studies indicated that other receptors are responsible for adenoviral gene transfer to the liver *in vivo*.

-101-

EXAMPLE 4

Description Of Adenoviral Vectors Containing A Fiber With Amino Acid Substitutions At The Heparin Sulfate Binding Domain In The Fiber Shaft

Vectors containing substitutions at all four of the amino acids in the
5 four amino acid motif in the Ad5 fiber shaft (residues 91 to 94, KKTK;
SEQ ID NO. 45) were generated in order to ablate the potential interaction
with HSP. The mutation is termed HSP because it potentially eliminates
binding to heparan sulfate proteoglycans. Vectors containing the HSP
mutation alone and combined with the KO1 mutation (fiber knob AB loop
10 mutation that ablates CAR binding), the PD1 mutation (penton mutation
that eliminates RGD/integrin interaction), and a triple knockout vector
(HSP, KO1, PD1) were generated.

Generation of the HSP fiber mutation: The HSP mutation was
incorporated into the fiber gene by using a PCR-based strategy of gene
15 splicing by overlap extension (PCR SOEing). First, a segment of the Ad5
genome extending from within the E3 region into the 5' end of the fiber
gene was amplified by PCR using the plasmid pSQ1 (Figure 3B) as a
template and two primers termed 5FF and 5HSPR. The DNA sequence of
5FF is as follows: 5' GAA CAG GAG GTG AGC TTA GA 3' (SEQ ID NO.
20 53). This sequence corresponds to base pairs 25,199 - 25,218 of pSQ1.
The DNA sequence of 5HSPR is as follows: 5' GGC TCC GGC TCC GAG
AGG TGG GCT CAC AGT GGT TAC ATT T 3' (SEQ ID NO. 64). 5HSPR
is a reverse primer for 5FF and corresponds to a region in the fiber shaft
adjacent to the KKTK (SEQ ID NO. 45) region. The primer contains a 5'
25 extension that encodes a GAGA substitution for the native KKTK
(encoded by SEQ ID NO. 45) amino acid sequence. A second PCR using
pSQ1 as a template amplified the region immediately 3' of the KKTK (SEQ
ID NO. 45) site and extending past the MunI site located 40 base pairs 3'
of the stop codon for the fiber gene. The two primers used for this

-102-

reaction were 3HSPF and 3FR. The DNA sequence of 3HSPF is as follows: 5' GGA GCC GGA GCC TCA AAC ATA AAC CTG GAA AT 3' (SEQ ID NO. 16). It contains a 5' extension that is complementary to the 5' extension of 5HSPR. The DNA sequence of 3FR is as follows: 5' GTG
5 GCA GGT TGA ATA CTA GG 3' (SEQ ID NO. 56).

The two PCR products were joined by PCR SOEing using primers 5FF and 3FR. The resulting PCR product was digested with the restriction enzymes XbaI and MunI. The 2355 bp fragment was gel purified and ligated with the 6477 bp XbaI to MunI fragment of the
10 plasmid pFBshuttle(EcoRI) (Figure 8) to generate the plasmid pFBSEHSP. The plasmid pFBshuttle(EcoRI) was generated by digesting the plasmid pSQ1 with EcoRI, then gel purifying and self-ligating the 8.8 kb fragment containing the fiber gene. Next, the fiber gene containing the HSP
15 mutation was transferred from pFBSEHSP into pSQ1 using a three-way ligation. The 16,431 bp EcoRI to NdeI fragment of pSQ1, the 9043 bp NdeI to XbaI fragment of pSQ1, and the 7571 bp XbaI to EcoRI fragment of pFBSEHSP were isolated and ligated to generate pSQ1HSP (Figure 9).

To generate a recombinant adenoviral vector containing the HSP mutation in the fiber gene, pSQ1HSP was digested with ClaI and
20 pAdmireRSVnBg (Figure 3A) was digested with Sall and PacI, then the two digested plasmids were co-transfected into 633 cells (von Seggern *et al.* (2000) *J Virology* 74:354-362). Homologous recombination between the two plasmids generated a full-length adenoviral genome capable of replication in 633 cells, which inducibly express Ad5 E1A and
25 constitutively express wild-type fiber protein. After propagation on 633 cells, the virus capsid contained wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP mutation, the viral preparation was used to infect PerC6 cells, which do

-103-

not express fiber. The resulting virus, termed Av1nBgFS*, was purified by standard CsCl centrifugation procedures.

Generation of vector containing the HSP and KO1 mutations

To generate an adenoviral vector containing the HSP and KO1 mutations in fiber, a PCR SOEing strategy identical to the one described above was used except that the plasmid pSQ1FKO1 was used as the template. The PCR SOEing product was digested with XbaI and MunI and ligated with the 6477 bp XbaI to MunI fragment of pFBshuttle(EcoRI) to generate pFBSEHSPKO1. The fiber gene containing the HSP and KO1 mutations was transferred from pFBSEHSPKO1 into the pSQ1 backbone using a three-way ligation strategy identical to the one described above for the HSP mutation alone, to generate the plasmid pSQ1HSPKO1 (Figure 10). Recombinant adenoviral vector containing the HSP and KO1 mutations in the fiber gene was generated by co-transfecting pSQ1HSPKO1 digested with ClaI and pAdmireRSVnBg digested with SalI and PacI into 633 cells. Adenovirus was propagated and purified as described above for the vector containing the HSP mutation alone. The resulting virus was termed Av1nBgFKO1S*.

Generation of vector containing the HSP and PD1 mutations

The following strategy was used to generate a recombinant adenoviral vector containing the fiber HSP mutation and the penton PD1 mutation. The plasmid pSQ1PD1 (Figure 4) was digested with the restriction enzymes Csp45I and SpeI and the 23,976 bp fragment was isolated and purified. In addition, the plasmid pSQ1HSP was also digested with Csp45I and SpeI and the 9090 bp fragment was isolated and purified and ligated to the 23,976 bp fragment to generate the plasmid pSQ1HSPPD1 (Figure 11), which contains the fiber HSP and penton PD1 mutations. An adenoviral vector was generated, propagated,

-104-

and purified as described above. The resulting virus was termed Av1nBgS*PD1.

Generation of vector containing the HSP, KO1, and PD1 mutations

To generate an adenoviral vector containing the HSP, KO1, and
5 PD1 mutations the following strategy was used. First, the plasmid
pSQ1PD1 was digested with Csp45I and SpeI and the 23,976 bp
fragment was isolated and purified. In addition, the plasmid
pSQ1HSPKO1 was digested with Csp45I and SpeI and the 9090 bp
fragment was isolated and purified. The two DNA fragments were ligated
10 to form the plasmid pSQ1HSPKO1PD1 (Figure 12). Recombinant
adenoviral vector was generated, propagated, and purified as described
above. The resulting virus was termed Av1nBgFKO1S*PD1.

EXAMPLE 5

***In Vitro* Evaluation Of Adenoviral Vectors Containing The HSP Fiber
15 Mutation**

The transduction efficiencies of adenoviral vectors containing the
HSP mutation in the fiber gene, either alone or combined with the KO1
and/or PD1 mutations, were evaluated on A549 and HeLa cells. The
transduction efficiencies were compared to that of Av1nBg, an adenoviral
20 vector containing wild type fiber and penton. The day prior to infection,
cells were seeded into 24-well plates at a density of approximately 1×10^5
cells per well. Immediately prior to infection, the exact number of
cells per well was determined by counting a representative well of cells.
Each of the vectors, Av1nBg (see, Stevenson *et al.* (1997) *J. Virol.*
25 71:4782-4790), Av1nBgS*, Av1nBgFKO1S*, Av1nBgS*PD1, and
Av1nBgFKO1S*PD1, were used to transduce A549 cells at each of the
following particle per cell (PPC) ratios: 100, 500, 1000, 2500, 5000,
10,000. HeLa cells were transduced with each of the above vectors, as
well as a vector containing the KO1 mutation alone (Av1nBgFKO1) and a

-105-

vector containing the PD1 mutation alone (Av1nBgPD1) at 2000 PPC. The cell monolayers were stained with X-gal 24 hours after infection and the percentage of cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done
5 in triplicate and three random fields in each well were counted, for a total of nine fields per vector.

The results (depicted in Figures 13A-13B) showed significantly reduced transduction efficiencies on A549 and HeLa cells using vectors containing the HSP mutation compared to Av1nBg. The vectors
10 containing the HSP mutations, however, demonstrated a dose response on A549 cells, in that increasing PPC ratios yielded increasing transduction.

Competition experiments were done to determine which receptor molecular interactions are involved in transduction of A549 cells by the
15 various vectors. Transductions were performed in the presence or absence of various competitors including Ad5 fiber knob, a 50 amino acid oligopeptide derived from Adenovirus serotype 2 penton base which spans the RGD tripeptide region, or heparin (Invitrogen Life Technologies, Gaithersburg, MD). Monolayers of A549 cells were cultured in Richters
20 medium supplemented with 10% FBS and were transduced with Av1nBg, Av1nBgS*, Av1nBgFKO1S*, Av1nBgS*PD1, or Av1nBgFKO1S*PD1 in infection medium (IM, Richters medium plus 2% FBS). Different PPC ratios were used for the different vectors to achieve measurable transduction levels. The PPC ratios were as follows: Av1nBg: 500 PPC,
25 Av1nBgS*: 10,000 PPC, Av1nBgFKO1S*: 20,000 PPC, Av1nBgS*PD1: 10,000 PPC, and Av1nBgFKO1S*PD1: 20,000 PPC. Fiber knob competition was performed by pre-incubating cells in IM containing 16 μ g/ml of fiber knob for 10 minutes at room temperature prior to infection with virus. Penton base peptide competition was performed by

-106-

- pre-incubating cells in IM containing 500nM peptide for 10 minutes at room temperature prior to infection with virus. Heparin competition was performed by pre-incubating each adenoviral vector in IM containing 3 mg/ml of heparin for 20 minutes at room temperature. In all cases, the competitor remained in the IM during the 1 hour infection when virus was rocked on the cell monolayers at 37° C in 5% CO₂. After infection, the monolayers were washed with PBS, 1 ml of complete medium was added per well and the cells were incubated for an additional 24 hours to allow for β -galactosidase expression. The cell monolayers were then fixed and stained with X-Gal. The percentage of cells transduced was determined by light microscopy as described above. Each condition was carried out in triplicate and three random fields per well were counted, for a total of nine fields per condition. The average percentage of transduction per high-power field was determined.
- The results of the competition experiment (Figure 13C) showed that fiber knob inhibited transduction of cells by all vectors except for those that contained the KO1 mutation. The penton base peptide only inhibited transduction by Av1nBgFKO1S*. Heparin inhibited transduction by Av1nBgFKO1S* and Av1nBgFKO1S*PD1, but did not affect transduction by any of the other viruses suggesting the presence of additional heparin binding sites on the adenoviral capsid but that the shaft contains the predominant site.

EXAMPLE 6

***In Vivo* Analysis Of Adenoviral Vectors Containing The HSP Mutation In Fiber**

The objective of this study was to evaluate the *in vivo* biodistribution of adenoviral vectors containing the HSP mutation and to determine whether this shaft modification influences adenoviral-mediated liver transduction. In addition, vectors containing the HSP mutation

-107-

combined with KO1, or PD1, or a combination of all three mutations were evaluated as well as vectors containing the KO1 mutation alone and the PD1 mutation alone. A positive control cohort received Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice
5 received each vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for
10 β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon real time PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β -galactosidase immunohistochemistry, hexon real-time PCR and
15 the chemiluminescent β -galactosidase activity assay were carried out as described in Example 3.

The results of the β -galactosidase activity assay (Figure 14A) and adenoviral hexon DNA content (Figure 14B) showed a dramatic reduction in liver transduction by vectors containing the HSP mutation. The vectors
20 containing the HSP mutation alone resulted in reducing adenoviral-mediated liver gene expression by approximately 20-fold. When combined with the KO1 mutation (HSP, KO1, PD1), yielded approximately a 1000-fold reduction in β -galactosidase activity in the liver compared to the control vector Av1nBg. The vector containing the KO1 mutation
25 alone showed a slight increase, on average, in liver transduction compared to Av1nBg, which is consistent with several previous experiments. The vectors containing the PD1 mutation alone or combined with KO1 showed a slight decrease in liver transduction compared to Av1nBg, although the decrease was not statistically significant. Analysis

-108-

of hepatic adenoviral hexon DNA content (Figure 14B) confirmed these results.

The results of the immunohistochemical staining of liver sections for β -galactosidase were consistent with the activity assays (data not shown) and demonstrated that gene expression was localized specifically to hepatocytes. Vectors containing the HSP mutation, either alone or in combination with KO1 and/or PD1, showed a dramatic reduction in hepatocyte transduction. The vector containing the KO1 mutation alone showed a slight increase in liver transduction as revealed by a more intense and frequent immunohistochemical staining pattern. The vectors containing the PD1 mutation, either alone or combined with KO1, showed little difference in transduction compared to Av1nBg.

EXAMPLE 7

Description of Adenoviral Vectors Containing the HSP Fiber Shaft Mutation with and without the KO1 Fiber Mutation and with and without a cRGD Targeting Ligand in the Fiber Knob HI Loop

Generation of vector containing the HSP fiber shaft mutation and a cRGD ligand in the HI loop: The following strategy was used to generate an adenoviral vector containing a fiber with the HSP shaft mutation and a cRGD ligand in the HI loop. The plasmid p5FloxHRFRGD was digested with the restriction enzymes BstXI and KpnI and the 1157 bp fragment was isolated and purified. In addition, the fiber shuttle plasmid pFBSEHSP, described in Example 1 above, was digested with BstXI and KpnI and the 4549 bp and 3156 bp fragments were isolated and purified. The three fragments were ligated to generate the plasmid pFBSEHSPRGD, which encodes a fiber containing the HSP mutation and cRGD in the HI loop. The fiber gene from this plasmid was transferred into the pSQ1 backbone as follows. The plasmid pFBSEHSPRGD was digested with EcoRI and XbaI and the 7601 bp fragment was isolated and purified. The plasmid pSQ1 (Figure 3B) was digested with the restriction enzymes

-109-

EcoRI, NdeI, and XbaI and the 16,431 bp EcoRI to NdeI fragment and the 9043 bp NdeI to XbaI fragment were isolated and purified. The three DNA fragments were ligated to generate the plasmid pSQ1HSPRGD (Figure 15A).

5 To generate a recombinant adenoviral vector containing the HSP mutation in the fiber gene along with a cRGD ligand in the HI loop, the plasmid pSQ1HSPRGD was digested with ClaI and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with SalI and PacI. After propagation on 633 cells, the virus capsid contained wildtype
10 and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP mutation and a cRGD ligand, the viral preparation was used to infect PerC6 cells, which do not express fiber. The resulting virus, termed Av1nBgS*RGD, was purified by standard CsCl centrifugation procedures.

15 **Generation of vector containing the HSP fiber shaft mutation, the KO1 fiber knob mutation, and a cRGD ligand in the HI loop**

 The following strategy was used to generate an adenoviral vector containing a fiber with the HSP shaft mutation, the KO1 fiber knob mutation, and a cRGD ligand in the HI loop. The plasmid p5FloxHRFRGD
20 was digested with the restriction enzymes BstXI and KpnI and the 1157 bp fragment was isolated and purified. In addition, the fiber shuttle plasmid pFBSEHSPKO1, described in Example 1 above, was digested with BstXI and KpnI and the 4549 bp and 3156 bp fragments were isolated and purified. The three fragments were ligated to generate the plasmid
25 pFBSEHSPKO1RGD, which encodes a fiber containing the HSP mutation, the KO1 mutation, and cRGD in the HI loop. The fiber gene from this plasmid was transferred into the pSQ1 backbone as follows. The plasmid pFBSEHSPKO1RGD was digested with EcoRI and XbaI and the 7601 bp fragment was isolated and purified. The plasmid pSQ1 (Figure 3B) was

-110-

digested with the restriction enzymes EcoRI, NdeI, and XbaI and the 16,431 bp EcoRI to NdeI fragment and the 9043 bp NdeI to XbaI fragment were isolated and purified. The three DNA fragments were ligated to generate the plasmid pSQ1HSPKO1RGD (Figure 15B).

5 To generate a recombinant adenoviral vector containing the HSP and KO1 mutations in the fiber gene along with a cRGD ligand in the HI loop, the plasmid pSQ1HSPKO1RGD was digested with ClaI and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with Sall and PacI. After propagation on 633 cells, the virus
10 capsid contained wildtype and mutant fiber proteins. To obtain viral particles containing only the modified fiber with the HSP and KO1 mutations and a cRGD ligand, the viral preparation was used to infect PerC6 cells, which do not express fiber. The resulting virus, termed Av1nBgFKO1S*RGD, was purified by standard CsCl centrifugation
15 procedures.

EXAMPLE 8

***In Vitro* Evaluation of Adenoviral Vectors Containing the HSP Fiber Shaft Mutation with or without the Fiber Knob KO1 Mutation and with or without a cRGD Ligand in the HI Loop**

20 The transduction efficiencies of adenoviral vectors containing the HSP fiber shaft mutation with or without the fiber KO1 mutation and with or without the cRGD ligand in the HI loop were evaluated on A549 cells. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing wild type fiber. The day prior to infection,
25 cells were seeded into 24-well plates at a density of approximately 1×10^5 cells per well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBgS*, Av1nBgFKO1S*,
Av1nBgS*RGD, and Av1nBgFKO1S*RGD, were used to transduce A549
30 cells at a particle to cell ratio of 6250. The cell monolayers were stained

-111-

with X-gal 24 hours after infection and the percentage of cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 16) showed that the cRGD ligand dramatically increased the transduction efficiencies of vectors containing the HSP mutation alone or combined with the KO1 mutation. Av1nBgS* yielded approximately 22% positive cells, while Av1nBgS*RGD yielded approximately 95% positive cells. Similarly, Av1nBgFKO1S* yielded only 4% positive cells, while Av1nBgFKO1S*RGD yielded 85% positive cells. Therefore, the vector containing the shaft mutation is viable and can be retargeted with the addition of a ligand.

EXAMPLE 9

Construction Of Ad5 Vectors Containing The Ad35 Fiber And Derivatives Thereof

The KO1 and HSP mutations in the Ad5 fiber protein (5F), described above, were designed to ablate interactions that are responsible for the normal tropism of the Ad5 virus. An alternative strategy to detarget the virus is to replace the Ad5 fiber with a fiber from another serotype which does not bind CAR and which does not possess the heparin sulfate proteoglycan (HSP) binding domain (KKTK; SEQ ID NO. 45) within the shaft. The fiber of adenovirus serotype 35 (35F) does not bind CAR and does not possess the HSP binding domain in its shaft. Replacement of the 5F with the 35F can detarget the liver and provide a suitable platform for retargeting the vector to the desired tissue.

Generation of an Ad5 based vector containing the Ad35 fiber: A PCR SOEing strategy was used to generate a vector based on the Ad5 serotype but containing the Ad35 fiber in place of the Ad5 fiber. First, PCR was used to amplify a region in the plasmid pSQ1 between the XbaI

-112-

site at bp 25,309 and the start of the fiber gene. The primers used for this reaction were P-0005/U and P-0006/L. The DNA sequence of P-0005/U was as follows: 5' C TCT AGA AAT GGA CGG AAT TAT TAC AG 3' (SEQ ID NO. 65). This sequence corresponds to bp 25,308
5 through 25,334 of pSQ1. The DNA sequence of P-0006/L was as follows: 5' TCT TGG TCA TCT GCA ACA ACA TGA AGA TAG TG 3' (SEQ ID NO. 66). It contains a 10 base pair 5' extension that is complementary to the start of the Ad35 fiber gene, while the remainder of the primer anneals to the sequence immediately 5' of the ATG start codon
10 of the fiber gene in pSQ1. A PCR product of the expected size, 583 bp, was obtained and the DNA was gel purified. A second PCR amplified the Ad35 fiber gene using DNA extracted from wildtype Ad35 virus as a template. The primers used for this reaction were P-0007/U and 35FMun. The DNA sequence of P-0007/U was as follows: 5' GT TGT
15 TGC AG ATG ACC AAG AGA GTC CGG CTC A 3' (SEQ ID NO. 67). It contains a 10 base pair 5' extension that is homologous to the 10 bp immediately prior to the ATG start codon of the fiber gene in Ad5. The remainder of the primer anneals to the start of the Ad35 fiber gene. The DNA sequence of 35FMun was as follows: 5' AG CAA TTG AAA AAT
20 AAA CAC GTT GAA ACA TAA CAC AAA CGA TTC TTT A GTT GTC GTC TTC TGT AAT GTA AGA A 3' (SEQ ID NO. 68). It contains a 46 base pair 5' extension that is complementary to the region of the Ad5 genome between the end of fiber and the MunI site 40 bp downstream of the fiber gene. In addition, the 5' extension encodes the last amino acid
25 and stop codon of the Ad5 fiber gene. This region was retained in the vector because it contains the polyadenylation site for the fiber gene. The remainder of the primer anneals to the 3' end of the Ad35 fiber gene, up to the next to last amino acid codon. A PCR product of the expected size, 1027 bp, was obtained and the DNA was gel purified. The two PCR

-113-

products were mixed and joined together by PCR SOEing using primers P-0005/U and P-0009. The DNA sequence of P-0009 was as follows: 5' AG CAA TTG AAA AAT AAA CAC GTT G 3' (SEQ ID NO. 69). It corresponds to bp 27,648 through 27,669 of pSQ1 and overlaps the

5 MunI site in that region. A PCR product of the expected size, 1590 bp, was obtained and gel purified. It was cloned into the plasmid pCR4blunt-TOPO (Invitrogen Corporation, Carlsbad CA) using the Zero Blunt TOPO PCR Cloning Kit from Invitrogen. This intermediate cloning step simplified DNA sequencing of the PCR SOEing product. The

10 resulting plasmid, termed pTOPOAd35F, was digested with XbaI and MunI and the 1585 bp digestion product was gel purified and ligated with the 6477 bp fragment of pFBshuttle(EcoRI) digested with XbaI and MunI to generate the plasmid pFBshuttleAd35F. The Ad35 fiber gene was transferred from pFBshuttleAd35F into pSQ1 as follows. The plasmid

15 pSQ1 was digested with EcoRI and the 24,213 bp fragment was gel purified. The plasmid pFBshuttleAd35F was linearized with EcoRI and ligated with the 24,213 bp fragment from pSQ1. Restriction diagnostics were performed to screen for constructs containing the Ad35 fiber gene inserted into the pSQ1 backbone in the correct orientation. The pSQ1

20 plasmid containing the Ad35 fiber gene in the proper orientation was termed pSQ1Ad35Fiber (Figure 17A). To generate adenoviral vector containing the Ad35 fiber, pSQ1Ad35Fiber was digested with ClaI and co-transfected into 633 cells with pAdmireRSVnBg which had been digested with SalI and PacI. After propagation on 633 cells, the resulting

25 virus contained Ad5 fiber and Ad35 fibers on its capsid. The virus was amplified on PerC6 cells to generate virus containing only the Ad35 fiber on its capsid. The resulting virus preparation was termed Av1nBg35F.

Construction of adenoviral vectors containing chimeric fibers derived from Ad5 and Ad35: Two chimeric fiber constructs were prepared

-114-

by PCR gene overlap extension using plasmids containing the full length Ad5 or Ad35 fiber cDNAs as templates. The Ad5 fiber tail and shaft regions (5TS; amino acids 1 to 403) were connected with the Ad35 fiber head region (35H; amino acids 137 to 323) to form the 5TS35H chimera, and the Ad35 fiber tail and shaft regions (35TS; amino acids 1 to 136) were connected with the Ad5 fiber head region (5H; amino acids 404 to 581) to form the 35TS5H chimera. The fusions were made at the conserved TLWT sequence at the fiber shaft-head junction.

For the construction of the 5TS35H chimera, the pFBshuttle(EcoR1) plasmid was used as the template with primers P1 and P2 to generate the 5' fragment. The 3' fragment was generated using the pFBshuttleAd35 plasmid as the template with the P3 and P4 primers. The sequence of each primer used in the construction of these chimeric fibers is listed in Table 2. Amplified PCR products of the expected size were obtained and were gel purified. A second PCR was carried out with the end primers P1 and P4 to join the two fragments together. The DNA fragment generated in the second PCR was digested with Xba1 and Mun1 and was cloned directly into pFBshuttle(EcoR1) to create the fiber shuttle plasmid pFBshuttle5TS35H.

TABLE 2
Primers Used For The Exchange Of Fiber Shaft Regions Between Ad5 And Ad35 Fibers

Primer designation	Sequence	SEQ ID
P1	5'-GAACAGGAGGTGAGCTTAGA-3'	70
P2	5'-GTTAGGTGGAGGGTTTATTCCGGTCCAC AAAGTTAGCTTATC-3'	71
P3	5'-GATAAGCTAACTTTGTGGACCGGAATAAA CCCTCCACCTAAC-3'	72
P4	5'-GTGGCAGGTTGAATACTAGG-3	73
P5	5'-GTTAGGAGATGGAGCTGGTGTAGTCCATA AGGTGTTAATAC-3'	74

-115-

Primer designation	Sequence	SEQ ID
P6	5'-GTATTAACACCTTATGGACTACACCAGCT CCATCTCCTAAC-3'	75
P7	5'-TGCGCAAAAACAATCACCACGACAATCACAAT GTACATTGGAAGAAATCATACG-3'	76
P8	5'-ACATTGTGATTGTCGTGGTGATT GTTTTTGCGCATATGCCATACAATTTGAATG-3'	77

5 For the construction of the 35TS5H chimera, the pFBshuttleAd35 plasmid was used as the template with the P1 and P5 primers to generate the 5' fragment. The 3' fragment was generated using the pFBshuttle(EcoR1) plasmid as the template with the P6 and P4 primers. Following the same procedure described above, the fiber shuttle plasmid

10 pFBshuttle35TS5H was generated.

For the 35TS5H and 5TS35H chimeras, the fiber gene was transferred from the pFBshuttle(EcoRI) backbone into pSQ1 as described above for the vector containing the Ad35 fiber. The resulting plasmids were called pSQ135T5H (Figure 18A) and pSQ15T35H (Figure 18B). In

15 addition, adenoviral vectors were generated using the co-transfection strategy described above.

Construction of Ad5 vectors containing the Ad35 fiber with a cRGD targeting peptide in the HI loop of the 35F fiber knob: To incorporate the cRGD targeting peptide into the Ad35 fiber HI loop, the

20 P7 and P8 oligonucleotide primers encoding the ten amino acid sequence HCDCRGDCFC (SEQ ID NO. 78) were synthesized. The pFBshuttleAd35 plasmid containing the full length Ad35 fiber cDNA was used as the template in the PCR reaction with the P1 and P7 primer pair or with the P4 and P8 primer pair in order to generate the 5' and 3' PCR fragments.

25 A second PCR was then carried out with the end primers P1 and P4 to join the two fragments together. The resulting PCR fragment was

-116-

digested with Xba1 and Mun1 and was cloned into pFBshuttle(EcoR1) to create the fiber shuttle plasmid pFBshuttleAd35cRGD. The modified Ad35 fiber gene was transferred into pSQ1 using the EcoRI cloning strategy described above to generate pSQ1Ad35FcRGD (Figure 17B).

- 5 Adenoviral vector was generated using the co-transfection strategy described above.

EXAMPLE 10

***In Vitro* Evaluation Of Adenoviral Vectors Containing 35F And Derivatives Thereof**

- 10 The transduction efficiencies of adenoviral vectors containing the 35F or derivatives thereof were evaluated on A549 cells. The transduction efficiencies were compared to that of Av1nBg, an adenoviral vector containing the 5F fiber. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1×10^5 cells per
- 15 well. Immediately prior to infection, the exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBg35F, Av1nBg5T35H and Av1nBg35T5H were used to transduce A549 cells from 0 up to 1,000 particle per cell (PPC) ratios. The cell monolayers were stained with X-gal 24 hours after
- 20 infection and the percentage of cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 19) showed similar transduction efficiencies on A549 cells using the
- 25 Av1nBg35F and Av1nBg5T35H vectors compared to Av1nBg. The Av1nBg35T5H showed much lower transduction efficiencies on A549 cells compared to Av1nBg as a result of the Ad35 shaft domain. The Ad35 shaft domain does not contain a HSP binding motif and the Av1nBg35T5H vector behaves similarly to the Av1nBgS* vector *in vitro*

-117-

and *in vivo*. These studies also demonstrate that vectors containing fiber proteins without an HSP binding site are fully viable.

EXAMPLE 11

5 *in Vivo* Evaluation Of Adenoviral Vectors Containing 35F And Derivatives Thereof

The objective of this study was to evaluate the *in vivo* biodistribution of adenoviral vectors containing 35F fibers and derivatives thereof to determine whether vectors containing these fibers ablate liver transduction due to their shaft regions. A positive control cohort received
10 Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median
15 lobe of the liver was placed in neutral buffered formalin to preserve the sample for β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β -
20 galactosidase immunohistochemistry, hexon real-time PCR and the chemiluminescent β -galactosidase activity assay were carried out as described in example 3.

The results of the β -galactosidase activity assay showed a dramatic reduction in liver transduction by vectors containing the Ad35 fiber or the
25 35T5H derivative (Figure 20) with an approximately 4- to 24-fold reduction in β -galactosidase activity in the liver compared to the control vector Av1nBg. These data demonstrate that shaft domains without HSP binding sites can effectively ablate hepatic *in vivo* gene transfer. In

-118-

particular, HSP is the major entry mechanism for liver *in vivo*. CAR binding is a minor entry pathway.

EXAMPLE 12

5 Construction Of Ad5 Vectors Containing The Ad Serotype 41 Short Fiber And Derivatives Thereof

The human adenovirus serotype 41 contains two different fibers on its capsid, encoded by two adjacent genes. One fiber has a molecular weight of 60kDa and is approximately 315A in length and is termed the long fiber. The other fiber has a molecular weight of 40kDa and is
10 approximately 250+ in length and is termed the short fiber. The Ad41 short fiber does not bind CAR and does not possess the heparin binding domain (KKTK) in its shaft. Therefore, this fiber provides a useful platform for adenoviral vector targeting.

Construction of adenoviral vectors based on Ad5 but containing the
15 Ad41 short fiber: A PCR SOEing strategy was used to generate a vector based on the Ad5 genome but containing the Ad41 short (Ad41s) fiber. First, PCR was used to amplify the region of pSQ1 between the XbaI site at bp 25,309 and the start of the fiber gene. The primer pair used for the PCR were P-0005/U and P-0010/L. The DNA sequence of P-0005/U was
20 as follows: 5' C TCT AGA AAT GGA CGG AAT TAT TAC AG 3' (SEQ ID NO. 65). The sequence corresponds to bp 25,308 through 25,334 of pSQ1 and overlaps the XbaI site in that region. The DNA sequence of P-0010/L was as follows: 5' TTC TTT TCA T CTG CAA CAA CAT GAA GAT AGT G 3' (SEQ ID NO. 79). It contains a 5' extension
25 corresponding to the first 10 bp of the Ad41s fiber gene. The remainder of the primer anneals to pSQ1 immediately 5' of the ATG start codon of the fiber gene. The PCR product was the expected size (583 bp). A second PCR was used to amplify the Ad41s fiber using the plasmid pDV60Ad41sF as a template. The primers used were P-0011/U and

-119-

P-0012/L. The DNA sequence of P-0011/U was as follows: 5' GT TGT TGC AG ATG AAA AGA ACC AGA ATT GAA G 3' (SEQ ID NO. 80). It contains a 10 bp 5' extension corresponding to the DNA sequence immediately 5' of the ATG start codon of the fiber gene in pSQ1. The remainder of the primer anneals to the beginning of the Ad41s fiber gene in pDV60Ad41sF. The DNA sequence of P-0012/L was as follows: 5' TG CAA TTG AAA AAT AAA CAC GTT GAA ACA TAA CAC AAA CGA TTC TTT ATT C TTC AGT TAT GTA GCA AAA TAC A 3' (SEQ ID NO. 81). It contains a 51 bp 5' extension corresponding to the sequence in pSQ1 from the last codon of the fiber gene through the MunI site 40 bp downstream of the fiber gene. The remainder of the primer anneals to the 3' end of the Ad41s fiber gene in pDV60Ad41sF. The PCR product was the expected size (1219 bp). The two PCR products were joined by PCR SOEing using primers P-0005/U and P-0009/L. The DNA sequence of P-0009/L was described above. The PCR SOEing reaction yielded the expected 1782 bp product. The product was cloned into pCR4blunt-TOPO to yield pCR4blunt-TOPOAd41sF. Next, pCR4blunt-TOPOAd41sF was digested with XbaI and MunI and the 1773 bp fragment containing the Ad41s fiber gene was gel purified. This fragment was ligated with the 6477 bp XbaI to MunI fragment of pFBshuttle(EcoRI) to generate pFBshuttleAd41sF. The Ad41s fiber gene was transferred into the pSQ1 backbone as follows. First, pFBshuttleAd41sF was linearized using EcoRI and this fragment was ligated with the 24,213 bp EcoRI fragment of pSQ1 to generate pSQ1Ad41sF (Figure 21A). Adenoviral vector containing the Ad41s fiber was generated using the co-transfection strategy described above.

Construction of Ad5 adenoviral vectors containing the Ad41 short fiber with a cRGD targeting ligand in the HI loop: A PCR SOEing strategy was used to generate a construct containing the Ad41s fiber with cRGD

-120-

in the HI loop. The plasmid pFBshuttleAd41sF was used as a template for the PCR amplifications. First, a 1782 bp fragment was amplified using primers 5FF and 41sRGDR. The primer 5FF was described above. It anneals to pFBshuttleAd41sF at the XbaI site upstream of the fiber gene.

5 The DNA sequence of the primer 41sRGDR was as follows: 5' AGT ACA AAA ACA ATC ACC ACG ACA ATC ACA GTT TAT CTC GTT GTA GAC GAC ACT GA 3' (SEQ ID NO. 82). It contains a 30 bp 5' extension that encodes the cRGD targeting ligand. The remainder of the primer anneals to pFBshuttleAd41sF from bp 2878 through 2903. A second PCR

10 amplified a 277bp region of pFBshuttleAd41sF using primers 3FR and 41sRGDF. The primer 3FR was described previously. It anneals to pFBshuttleAd41sF at the MunI site downstream of the fiber gene. The DNA sequence of 41sRGDF was as follows: 5' TGT GAT TGT CGT GGT GAT TGT TTT TGT ACT AGT GGG TAT GCT TTT ACT TTT 3' (SEQ ID

15 NO. 83). It contains a 30 bp 5' extension that encodes the cRGD targeting ligand and is complementary to the extension on 41sRGDR. The remainder of the primer anneals to pFBshuttleAd41sF from bp 2904 through 2924. The two PCR products were joined by PCR SOEing to generate a 2059 bp fragment using primers 5FF and 3FR. The product

20 was digested with XbaI and MunI and the 1803 bp DNA fragment was gel purified. The fragment was ligated with the 6477 bp fragment resulting from digestion of pFBshuttle(EcoRI) with XbaI and MunI. The resulting plasmid was termed pFBshuttleAd41sRGD. This plasmid was linearized by EcoRI digestion and ligated with the 24,213bp EcoRI

25 fragment of pSQ1 to generate pSQ1Ad41sRGD (Figure 21B).

-121-

EXAMPLE 13

***In Vivo* Evaluation Of Ad5 Vectors Containing The Ad41 Short Fiber And Derivatives Thereof**

- This example evaluates the *in vivo* biodistribution of adenoviral
- 5 vectors containing 41sF fibers and derivatives thereof to determine whether vectors containing the these fibers ablate liver transduction due to modified shaft regions. A positive control cohort received Av3nBg (see, Gorziglia *et al.* (1996) *J. Virology* 70:4173-4178) or Ad5. β Gal. Δ F/5F, and a negative control group received HBSS.
- 10 Ad5. β Gal. Δ F/5F is a derivative of the fiberless vector Ad5. β gal. Δ F (ATCC accession number VR2636) modified to express AD5 fiber (see, *e.g.*, International PCT application No. WO 01/83729).

- The Ad5. β Gal. Δ F vector was pseudotyped with the Ad41sF fiber protein and injected *in vivo*. Cohorts of five C57BL/6 mice received each
- 15 vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. The median lobe of the liver was placed in neutral buffered formalin to preserve the sample for
- 20 β -galactosidase immunohistochemistry. In addition, tissue from each organ was frozen to preserve it for hexon PCR analysis to determine vector content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. β -galactosidase immunohistochemistry, hexon real-time PCR and the
- 25 chemiluminescent β -galactosidase activity assay was carried out as described in Example 3.

The results of the hexon DNA analysis showed a dramatic reduction in liver transduction by vectors containing the Ad41sF fiber

-122-

(Figure 22) with an approximately a 5-fold reduction in liver adenoviral DNA content compared to either control vector.

In the above examples, several novel adenoviral vectors were generated containing various fiber modifications designed to ablate the normal tropism of the vector (see Table 3). Vectors were generated in which the heparan sulfate binding domain in the fiber shaft was replaced by amino acid substitutions. This mutation, termed HSP, was also combined with the KO1 mutation (fiber knob AB loop mutation that ablates CAR binding), and the PD1 mutation (penton mutation that eliminates RGD/integrin interaction). In addition, a vector containing all three mutations (HSP, KO1, PD1) was generated. All vectors containing the HSP mutation, either alone or combined with other capsid modifications, showed dramatically reduced transduction efficiencies on A549 and HeLa cells. Furthermore, the same vectors showed dramatically reduced transduction of the liver following systemic delivery to mice. As an alternative strategy to ablate the normal tropism of Ad5-based vectors, the Ad5 fiber was replaced by a fiber from a different adenovirus serotype which does not bind CAR and does not contain the heparan binding domain in the shaft. Thus, vectors were generated containing the Ad35 fiber and the Ad41 short fiber. Versions of these two vectors containing a cRGD targeting ligand in the HI loop of the fiber were also produced. Additionally, vectors containing chimeric fibers were generated. A vector containing the Ad35 fiber tail and shaft regions fused to the Ad5 fiber knob domain as well as a vector containing the Ad5 fiber tail and shaft fused to the Ad35 fiber knob domain were constructed. Vectors containing either the entire Ad35 or Ad41 short fiber showed a significant reduction in liver transduction following delivery to mice via the tail vein. The observation of reduced liver transduction using vectors containing either an HSP mutation, the Ad35 fiber, or the

-123-

Ad41 short fiber indicates the feasibility of detargeting adenoviral vectors *in vivo*. *In vitro* data with the Ad35 fiber or the Ad41 short fiber with cRGD (see Example 14) indicate that the virus is completely viable, that is, it is not damaged by the absence of an HSP binding site and is

5 retargetable. Taken together these data suggest that these vectors provide a suitable platform for retargeting strategies.

TABLE 3
Description Of Recombinant Adenoviral Vectors Used
To Demonstrate That Shaft Modifications Influence Tropism *In Vivo*
Vector

10

Vector	Description
Av1nBg	An E1 and E3-deleted adenoviral vector encoding a nuclear localizing β -galactosidase
Ad5 Fiber derivatives:	
Av1nBgFKO1	The same as Av1nBg but containing the KO1 AB loop mutation in the fiber gene
15 Av1nBgPD1	The same as Av1nBg but containing the penton PD1 mutation that deletes the integrin binding, RGD tripeptide
Av1nBgS*	The same as Av1nBg but containing the 4 amino acid substitution in the shaft referred to as S* that modifies the HSP binding motif
Av1nBgFKO1S*	The same as Av1nBg but containing the fiber KO1 and S* mutations combined
Av1nBgS*PD1	The same as Av1nBg but containing the fiber S* and penton PD1 mutations combined
Av1nBgFKO1S*PD1	The same as Av1nBg but containing the fiber KO1, S* and penton PD1 mutations combined
Ad35 fiber derivatives:	
20 Av1nBg35F	The same as Av1nBg but containing the full length Ad35 fiber cDNA
Av1nBg5T35H	The same as Av1nBg but containing the 5T35H chimeric fiber
Av1nBg35T5H	The same as Av1nBg but containing the 35T5H chimeric fiber
Av1nBg35FRGD	The same as Av1nBg but containing the full length Ad35 fiber cDNA with a cRGD ligand in the HI loop of the Ad35 fiber
25 Ad41sF fiber derivatives:	

-124-

Vector	Description
Av1nBg41sF	The same as Av1nBg but containing the full length Ad41 short fiber cDNA
Av1nBg41sFRGD	The same as Av1nBg but containing the full length Ad41 short fiber cDNA with a cRGD ligand in the HI loop of the Ad41 short fiber

EXAMPLE 14**5 *In Vitro* Evaluation Of Adenoviral Vectors Containing The Ad41sF With A cRGD Ligand In The HI Loop**

- The transduction efficiencies of adenoviral vectors containing the Ad41sF fiber with the cRGD ligand in the HI loop were evaluated on A549 cells. The transduction efficiencies were compared to that of
- 10** Av1nBg, an adenoviral vector containing wild type fiber or Av1nBgFKO1RGD, an adenoviral vector containing the KO1 mutation in combination with the cRGD ligand in the HI loop. The day prior to infection, cells were seeded into 24-well plates at a density of approximately 1×10^5 cells per well. Immediately prior to infection, the
- 15** exact number of cells per well was determined by counting a representative well of cells. Each of the vectors, Av1nBg, Av1nBgFKO1RGD, and Av1nBg41sFRGD were used to transduce A549 cells at a particle to cell ratios of 0 up to 10,000. The cell monolayers were stained with X-gal 24 hours after infection and the percentage of
- 20** cells expressing β -galactosidase was determined by microscopic observation and counting of cells. Transductions were done in triplicate and three random fields in each well were counted, for a total of nine fields per vector. The results (Figure 23) show that the Av1nBg41sFRGD vector transduced cells to an equivalent level as Av1nBgFKO1RGD at all
- 25** vector doses examined. Neither FKO1 or Ad41sF can bind CAR. The Ad41sF does not normally interact with CAR and additionally does not contain the HSP binding motif within the shaft domain. These data show

-125-

that targeting peptides inserted into the loop regions of the fiber knob of KO1 and Ad41sF allows for transduction of target cells via the targeted receptor. Surprisingly, HSP, not CAR and integrins, is the major entry route *in vivo* and ablation of HSP binding permits targeting of adenoviral
5 vectors.

EXAMPLE 15

Effect of the shaft modification on the biodistribution of adenoviral vectors *in vivo*

The influence of fiber and penton modifications on the *in vivo*
10 biodistribution of adenoviral vectors containing fiber head, shaft and penton mutations was examined. Vectors containing the HSP mutation combined with KO1, or PD1, or a combination of all three mutations were evaluated as well as vectors containing the KO1 mutation alone and the PD1 mutation alone. The indicated adenoviral vectors were systemically
15 administered to C57BL6 mice as described above. A positive control cohort received Av1nBg and a negative control group received HBSS. Cohorts of five C57BL/6 mice received each vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed approximately 72 hours after vector administration by carbon dioxide
20 asphyxiation. Liver, heart, lung, spleen, and kidney were collected from each animal. Tissue from each organ was frozen to preserve it for real time PCR analysis to determine adenoviral hexon DNA content. A separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. Hexon real-time PCR
25 and the chemiluminescent β -galactosidase activity assay was carried out as described in Example 3.

The results derived from the liver are described in Example 6 (Figure 14A and B) and also shown in Figure 26 with results presented as percent control of Av1nBg. The effect of the S* shaft modification on

-126-

the biodistribution of adenovirus to the other organs is shown in Figure 25. The average adenoviral DNA content was determined as adenoviral genomic copies per cell and expressed as a percentage of the Av1nBg (+) control value. The average percent control value + standard deviation is shown (n = 5 per group) for each tissue examined (Figure 25).

Systemic delivery of Ad5 based vectors with wild-type fiber results in a preferential accumulation of vector DNA in the liver with 64 copies per cell with significantly less DNA found in the other organs with 1.32 copies per cell found in lung, 2.18 copies per cell in spleen, 0.47 copies per cell found in heart, and 0.72 copies per cell in the kidney. All differences found with PD1, S*, KO1PD1, KO1S*, S*PD1, and KO1S*PD1 were significantly different than the Av1nBg (+) control using a unpaired, t-test analysis, P value (0.024. When expressed as a percent of the Av1nBg control values, the influence of each mutation, individually or in combination, becomes apparent. The S* mutation dramatically reduced gene transfer to all four organs, whereas, the KO1 mutation did not. Thus, the importance of the shaft for transduction *in vivo* extends to organs besides the liver. Finally, gene transfer to the lung, heart, and kidney was diminished with PD1 suggesting a role for integrin binding in vector entry in these organs.

EXAMPLE 16

Retargeting the S*, shaft modification and the 41sF fiber *in vivo*

Vectors containing the HSP mutation have been shown to effectively detarget adenoviral vectors *in vivo* (see examples 6 and 15). The objective of this study was to evaluate the ability to retarget vectors containing the S* modification or the Ad41sF to tumors *in vivo*. A cRGD peptide was genetically incorporated into the fiber HI loop and evaluated *in vitro* (Examples 8 and 14). These same vectors were then evaluated *in vivo* in tumor-bearing mice. Athymic nu/nu female mice were injected

-127-

with 8×10^6 A549 cells on the right hind flank. When tumors reached approximately 100mm³ in size, they were randomized into treatment groups. Cohorts of 6 mice received each vector via tail vein injection at a dose of 1×10^{13} particles per kg. The animals were sacrificed

- 5 approximately 72 hours after vector administration by carbon dioxide asphyxiation. Tumor, liver, heart, lung, spleen, and kidney were collected from each animal. Tissue from each organ was frozen to preserve it for real time PCR analysis to determine adenoviral hexon DNA content. Hexon real-time PCR was carried out as described in example 3. A
- 10 separate sample of liver from each mouse was frozen to preserve it for a chemiluminescent β -galactosidase activity assay. Hexon real-time PCR and the chemiluminescent β -galactosidase activity assay was carried out as described in example 3.

- The adenoviral vector biodistribution to the liver and tumor for each
- 15 treatment group is shown in Figure 27. Vectors containing the S*, KO1S*, and 41sF fibers effectively detargeted the liver and tumor resulting in a significant reduction in the amount of adenoviral DNA found in each tissue in comparison to the Av1nBg control. Vectors containing the cRGD targeting ligand restored transduction of the tumors to levels
- 20 comparable to that achieved with the untargeted vector.

- These data demonstrate successful liver detargeting accompanied with tumor retargeting. The extent of tumor retargeting is relates to the affinity and type of ligand that is used. These data demonstrate the successful development of a targeted, systemically deliverable adenoviral
- 25 vector that will target tumors *in vivo*.

-128-

EXAMPLE 17**Scale-Up Method For The Propagation Of Detargeted Adenoviral Vectors**

The growth and propagation of doubly or triply ablated adenoviral vectors requires novel scale up technologies. These detargeted vectors
5 require alternative cellular entry strategies to allow for the efficient growth and generation of high titer preparations. A strategy for vector growth that is generally applicable to all detargeted adenoviral vectors, that does not require the development of new cell lines, and that also can be used for generating targeted vectors is provided herein.

10 Three recombinant adenoviral vectors were prepared that contain single mutations in the fiber or penton or both mutations combined into one vector. These vectors are designated Av3nBgFKO1, Av1nBgPD1, and Av1nBgFKO1PD1, respectively. The construction of these vectors is described above and a general description of each vector can be found in
15 Table 1 above.

Scale-up of detargeted adenoviral vectors: A polycation, specifically hexadimethrine bromide was obtained from Sigma Chemical Co (St. Louis, MO), Catalog No. 52495, and was maintained in the medium at 4 μ g/ml during the course of transfections and infections. To illustrate the affects
20 of hexadimethrine bromide on the yield of detargeted adenoviral vectors the following experiment was carried out. Seven plates of AE1-2a adenoviral producer cells (Gorziglia *et al.* (1996) *J. Virology* 70:4173-4178) were transduced with 10 particles per cells of each of the indicated vectors (See Table 4). Each vector was incubated with medium
25 (Richters with 2% HI-FBS) containing hexadimethrine bromide at 4 μ g/ml for 30 min at room temperature prior to infection. The infection was carried out for 2 hrs. Complete medium containing hexadimethrine bromide at 4 μ g/ml was added to each plate. Final concentration of hexadimethrine bromide in all of these experiments was maintained at

-129-

4 $\mu\text{g/ml}$. The titers were determined spectrophotometrically using the conversion of 1OD at A260nm per 1×10^{12} particles (Mittereder *et al.* (1996) *J Virology* 70:7498-7509). The total particle yield was then normalized for the number of plates used for transduction.

- 5 The inclusion of hexadimethrine bromide in the medium during the course of infection allows for the efficient propagation of detargeted adenoviral vectors containing fiber and penton mutations either alone or in combination. The effect of hexadimethrine bromide on vector yields is shown in Table 4. A 35-fold improvement in the yield of Av3nBgFKO1
- 10 was found when hexadimethrine bromide was included in the culture medium and resulted in increasing the vector yield from 1.3×10^{10} up to 4.6×10^{11} vector particle per plate. Hexadimethrine bromide has a minimal effect on the yield of the Av1nBgPD1 adenoviral vector containing the penton, PD1 mutation with only a 1.2 fold improvement.
- 15 The greatest effect using hexadimethrine bromide was found on the propagation of the doubly ablated adenoviral vector, Av1nBgFKO1PD1 with increases in vector yield from barely detectable levels up to 4.53×10^{10} vector particles per plate. These data demonstrate that use of nonspecific entry mechanisms allows for the efficient scale-up of
- 20 detargeted adenoviral vectors.

TABLE 4
Efficient Scale-Up Of Detargeted Adenoviral Vectors Using
hexadimethrine bromide

25 Vector	Vector Yield (particles/plate)		Fold Improvement
	(-) hexadimethrine bromide	(+) hexadimethrine bromide	
Av1nBg	3.89×10^{11}	5.72×10^{11}	1.47
Av3nBg	8.58×10^{10}	2.38×10^{11}	2.77
Av3nBgFKO1	1.30×10^{10}	4.60×10^{11}	35.4
Av1nBgPD1	1.95×10^{11}	2.40×10^{11}	1.23

-130-

Vector	Vector Yield (particles/plate)		Fold Improvement
	(-) hexadimethrine bromide	(+) hexadimethrine bromide	
Av1nBgFKO1PD1	TLTC*	4.53×10^{10}	†

*TLTC: Too low to count, a faint virus band was collected and the particle concentration was too dilute for titer determination.

5 † Significant improvement

The use of alternative polycations including protamine sulfate and poly-lysine as well as bifunctional proteins such as the anti-penton:TNF α fusion protein was investigated. Figure 24 show results that demonstrate all the reagents tested had some effect on enhancing transduction of the

10 Av3nBgFKO1 vector. All of these compounds, when maintained in the medium during infection, enhanced transduction of the Av3nBgFKO1 detargeted adenoviral vector.

Bifunctional reagents:. The use of bifunctional reagents for the propagation of detargeted adenoviral vectors was examined using the

15 anti-penton:TNF α fusion protein. This particular reagent is a fusion protein between an antibody against Ad5 penton and the TNF α protein that is produced using stably transfected insect cells. This reagent will bind specifically to the adenoviral capsid via penton base and allow for binding to cell surface TNF receptors. The use of this reagent for the propagation

20 of detargeted vectors is illustrated in Table 5 using Av3nBgFKO1 (also shown in Figure 24). Monolayers of S8 cells were infected with 10 or 100 particles per cell of Av3nBgFKO1 or a control vector in the presence or absence of 1 μ g/ml of the anti-penton:TNF α fusion protein. The monolayers were visually inspected over time for vector spread as

25 indicated by the extent of cytopathic effect (CPE). The percentage of CPE at each time point is shown. The use of this bifunctional reagent

-131-

clearly enhances the spread of the Av3nBgFKO1 vector throughout the monolayer.

TABLE 5
Efficient Scale-Up Of Detargeted Adenoviral
Vectors Using Bifunctional Reagents: Anti-Penton:TNF α

5

	10 ppc - anti-penton TNF	10 ppc + anti-penton TNF	100 ppc - anti-penton TNF	100 ppc + anti-penton TNF
	Percentage of CPE			
	Ad5Luc1			
10	24 h	0%	0%	0%
	48 h	20-30%	20-30%	90-100%
	72 h	60-70%	80-90%	100%
	120 h	100%	100%	100%
	Av3nBgKO1 24hrs			
15	24 h	0%	0%	0%
	48 h	0%	10-20%	90-100%
	72 h	5%	60-70%	100%
	120 h	40-50%	100%	100%

20

EXAMPLE 18

This Example and the following Example describe construction of adenoviral Ad5 particles that express heterologous fibers. The fibers are modified at the N-terminus to increase incorporation into the Ad5 particle. The N-terminus, typically, the first at least 16 or 17 amino acids is

25

modified so that the sequence resembles the Ad5 terminus.

Expression of Fiber Proteins from Subgroups B, C and D of Human Adenovirus

Constructs for expression of fibers from several adenoviral serotypes including, Types 16, 30, and 35 (subgroup B), and Types 19p and 37 (subgroup D), were generated.

30

-132-

Construction of Ad37, Ad19p and Ad30 fiber expression plasmids

The open reading frames (ORFs) of Ad37 (SEQ ID NO. 31), Ad19p (SEQ ID NO. 33) or Ad30 (SEQ ID NO. 35) fiber proteins were PCR amplified using the following primers:

- 5 Forward primer (L37): TGT CTT **GGA TCC** AAG ATG AAG CGC
GCC CGC CCC AGC GAA GAT GAC TTC (SEQ ID NO. 84)
Reverse primer (37FR): AAA CAC **GGC GGC CGC** TCT TTC ATT
CTT G (SEQ ID NO. 85)

- Primers L37 and 37FR include BamHI and NotI sites (in bold),
10 respectively, to facilitate subcloning. In addition, primer L37 introduces mutations into the 5' end of each fiber protein so that the resulting fiber proteins more closely resemble the Ad5 fiber N-terminal sequence for assembly onto Ad5 particles. For Ad37, the native N-terminus and modified N-terminus have the following amino acid sequences:

- 15 Native Ad37 N-terminus: MSKRLRVE (SEQ ID NO. 86)
Modified Ad37 N-terminus: MKRARPSE (SEQ ID NO. 87)

- The amplified fibers were cloned into the BamHI and NotI sites of plasmid pCDNA3.1zeo(+) (Invitrogen). The Ad5 tripartite leader (TPL) sequence from plasmid pDV55 (see EXAMPLE 20 and SEQ ID NO. 88),
20 which is flanked by BamHI sites (SEQ ID NO. 88), was then subcloned into the BamHI site of each fiber expression plasmid to generate plasmids pDV121 (Ad37), pDV145 (Ad19p) and pDV164 (Ad30). Construction of plasmid pDV55 is set forth in EXAMPLE 20 (see also, copending U.S. application Serial No. 09/482,682, also filed as International PCT
25 application No. PCT/US00/00265; and in U.S. application Serial No. 09/562,934, also filed as International PCT application No. PCT/EP01/04863. The combination of the CMV promoter present in pCDNA3.1zeo(+) and the addition of the TPL sequence from pDV55 (SEQ ID NO. 88) provides for high-level expression of viral proteins.

-133-

Construction of Ad16 and Ad35 fiber expression plasmids

Ad16 and Ad35 fiber expression plasmids were generated in a similar manner with the following modifications. To PCR amplify Ad16 (SEQ ID NO. 37) and Ad35 (SEQ ID NO. 39) fiber, the forward primer
5 was designed to incorporate an NdeI site (in bold), which is present at nucleotide 48 of Ad5 fiber (SEQ ID NO. 1), but absent in Ad16 and Ad35 fiber sequences. The reverse primers contained a NotI site (in bold):

Ad16/Ad35 forward primer (F16 5'): CCG GTC TAC **CCA TAT**
GAA GATG (SEQ ID NO. 89)

10 Ad16 reverse primer (F16 3'): TGG TGC **GGC CGC** TCA GTC ATC
TTC TCTG (SEQ ID NO. 90)

Ad35 reverse primer (F35 3'): TGG TGC **GGC CGC** TTA GTT GTC
GTC TTC TGT AAT G (SEQ ID NO. 91)

The Ad16 and Ad35 PCR products were cloned into the NdeI and
15 NotI sites of pCDNA3.1zeo(+), resulting in plasmids pDV147 (Ad16) and pDV165 (Ad35). The NdeI site of pCDNA3.1zeo(+) is within the CMV promoter region of the plasmid, therefore, the resulting plasmids lacked the 3' portion of the CMV promoter region. In addition, the inserted fiber sequences were lacking the portion of the fiber sequence that is 5' to the
20 engineered NdeI site. To insert the necessary regulatory sequences and N-terminal fiber sequence, plasmid pDV67 (described in Example 22 and also U.S. Application Serial No. 09/562,934, and is available from the ATCC under accession number PTA-1145) was digested with NdeI to remove a fragment that contains the 3' portion of the CMV promoter, the
25 complete Ad5 TPL sequence and the 5' portion of the Ad5 fiber sequence. The NdeI fragment was subcloned into plasmids pDV147 and pDV165 to generate the complete Ad16 and Ad35 expression plasmids, pDV156 and pDV166, respectively. Expression of these constructs results in chimeric fiber proteins containing the 17 N-terminal amino acids

-134-

from Ad5 fiber (see SEQ ID NO. 2) and the remainder of the fiber sequence from either Ad16 or Ad35. The nucleotide sequences of the chimeric fibers are listed in SEQ ID NO. 41 (Ad5/Ad16) and SEQ ID NO. 43 (Ad5/Ad35).

5 Expression and trimerization of recombinant Ad fiber proteins

To verify expression and trimerization of the recombinant proteins, the resulting plasmids were transfected into 293T cells, which are identical to 293 cells except they express an integrated SV40 large T antigen gene. 239 cells are an adenovirus-transformed human embryonic
10 kidney cell line obtained from the ATCC, where they are deposited under Accession Number CRL 1573. 293T cells express CAR and α_v integrins. Fiber expression was detected by immunoblotting of cell lysates using the 4D2 monoclonal antibody (Research Diagnostics Inc., Flanders, N.J.), which recognizes an epitope conserved among fibers of different
15 serotypes. To generate stable cell lines, constructs were electroporated into an A549-derived cell line that complements the Ad E1a and E2a functions (Gorziglia *et al.*, *J. Virol.* 70:4173-4178 (1996)) and stable clones were derived by selection with zeocin. Clones that expressed high levels of the fiber protein were identified by immunoblotting with the 4D2
20 antibody.

Generation of Adenovirus Particles Pseudotyped with Subgroup B, C or D Ad Fiber Protein

A system for producing Ad vector particles with different or
25 modified fiber proteins, that therefore have altered tropism (pseudotyping) is known (see, *e.g.*, Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000); Wu *et al.*, *Virology* 279:78-89 (2001)). Briefly, an E1-deleted Ad vector is modified by further deletion of the fiber gene, such that the virus produces no fiber protein. Growth of the fiber-deleted viruses in

-135-

packaging cells that express a fiber protein as well as complementing the E1 deletion allows generation of particles with any desired fiber.

Packaging cell lines were generated by stably transfecting expression constructs for the fibers of interest (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)) into an A549-derived E1- and E2a-complementing cell line (Gorziglia *et al.*, *J. Virol.* 70:4173-4178 (1996)), and clones that expressed the fibers at high levels were selected. The resulting lines complement E1 and fiber deletions, and were used to propagate Ad5.GFP.ΔF, a fiber-deleted Ad5 vector with a GFP transgene in place of the deleted E1 sequences (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). The particles produced by growth in the various cell lines are identical except for their fiber proteins. Viral particles were isolated by CsCl gradient centrifugation, and assayed for the presence of fiber by immunoblotting using monoclonal Ab 4D2. As a control for equal loading, the blot was re-probed with a polyclonal antibody against the Ad penton base protein. All recombinant fibers were capable of assembly onto Ad5 particles.

EXAMPLE 19

Construction and Propagation of Adenovirus Particles with Genomic Fiber Substitutions

The fiber-deletion system described in Example 18 allows rapid evaluation of fiber proteins for their infectious properties. The resulting particles produced are less infectious than the corresponding first-generation vectors (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). Therefore, viral backbones with the subgroup B, C or D fibers substituted in place of the Ad5 fiber gene were constructed. To facilitate construction of these vectors, the AdEasy system (see, U.S. Patent No. 5,922,576; see, also He *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:2509-2514; the system is publicly available from the authors and

-136-

other sources) was modified. This system includes a large plasmid (pAdEasy) that contains most of the Ad5 genome and smaller shuttle plasmids with the left end of the viral genome, including an E1 deletion and polylinker for insertion of transgenes. Recombination between
5 pAdEasy and a shuttle plasmid in *E. coli* reconstitutes a full-length infectious Ad genome. All plasmids used were derivatives of pAdEasy1 with different fiber proteins substituted in place of the Ad5 fiber.

Construction of pDV153

p5FloxHRF (SEQ ID NO. 92) contains the right end of the Ad5
10 genome with a PacI site in place of the right ITR. There is a unique (naturally occurring in Ad5) MunI site approximately 30 nucleotides downstream of the fiber stop codon. To facilitate fiber substitutions, this site was moved to lie immediately downstream of the fiber ORF. This preserved the sequence around the fiber gene and its spacing relative to
15 the other adenovirus genes.

The oligos MunITOP (AAT TGT GTT ATG TTT AAA CGT GTT TAT TTT TG; SEQ ID NO. 93) and MunIBOTTOM (AAT TCA AAA ATA AAC ACG TTT AAA CAT AAC AC; SEQ ID NO. 94) were annealed and ligated into the unique MunI site of p5FloxHRF to generate plasmid
20 pDV153. Insertion of the oligo destroyed the original MunI site by changing one base at its 5' end, but resulted in insertion of a new MunI site that is 32 base pairs closer to the fiber ORF than the original MunI site.

Construction of an Ad vector with a chimeric Ad5/Ad37 fiber gene

25 To replace the Ad5 fiber sequence of pDV153 with Ad37 fiber, pDV153 was digested with SphI and MunI. This removed all but the N-terminal 183 nucleotides of Ad5 fiber (see SEQ ID NO. 1). Ad37 fiber was then PCR amplified using a 5' primer (F37 5'SphI) TAC CAA TGG CAT GCT ATC CCT CAA GG (SEQ ID NO. 95) that added a SphI site and

-137-

a 3' primer (F37 3'EcoRI) AAA CAC GGG AAT TCG TCT TTC ATT C (SEQ ID NO. 96) that added an EcoRI restriction site. The 3' primer was designed to have an EcoRI site since the Ad37 fiber sequence contains a MunI restriction site. The nucleotide overhangs left by digestion with
5 EcoRI and MunI are compatible, allowing the PCR products to be cloned into pDV153 digested with SphI and MunI. This resulted in expression of a chimeric Ad5/Ad37 fiber protein with the N-terminal 61 amino acids from Ad5 fiber (SEQ ID NO. 2) and the remainder of the protein from Ad37 (corresponding to amino acid 62 to the end of Ad37 fiber; SEQ ID
10 NO. 32).

After Ad37 fiber was ligated into pDV153, the SpeI/PacI fragment was used to replace the SpeI/PacI fragment of pAdEasy, resulting in plasmid pDV158. Plasmid pDV158 was then recombined with the shuttle plasmid pAdTrack, which contains a CMV-driven EGFP reporter gene (He
15 *et al.*, *Proc. Natl. Acad. Sci. USA* 95:2509-2514 (1998); U.S. Patent Serial No. 5,922,576). The resulting Ad vector (Ad5.GFP.37F) has the EGFP reporter at the site of the E1 deletion and the chimeric Ad5/Ad37 fiber gene in the viral chromosome, and infects cells via the Ad37 receptor rather than CAR. pDV158 can be readily used to create
20 adenovirus particles with the same fiber protein but different transgenes.

Propagation of Ad5.GFP.37F in 633 cells

The Ad5.GFP.37F genome is infectious, and readily begins replicating as a virus. Since the 293 cells (ATCC Accession No. CRL
25 1573) normally used for Ad propagation do not express high levels of the Ad37 receptor, this virus does not efficiently propagate. To facilitate viral amplification, stocks of the virus were maintained in the 633 cell line (ATCC Accession No. PTA-1145), which expresses a wildtype Ad5 fiber protein (Von Seggern *et al.*, *J. Virol.* 74:354-362 (2000)). The particles
30 therefore contain the Ad5 fiber produced by the cells and the chimeric

-138-

Ad5/Ad37 fiber protein encoded by the virus. The Ad5 fiber allows the virus to re-infect the cell lines used for viral growth. A final round of growth in 293 cells (which do not express a fiber protein) generates particles with only the vector-encoded Ad37 fiber. To assess Ad5 and

5 Ad37 fiber content of Ad5.GFP.37F particles, viral particles produced in either 633 cells or 293 cells were immunoblotted with anti-fiber monoclonal Ab 4D2. 633-grown particles contained the Ad5 and Ad5/37 fibers, while virus produced in 293 cells contained only the Ad5/37 chimeric fiber. Particles of the first-generation Ad vector Ad5. β gal.wt,

10 which contain only the wildtype Ad5 fiber (Wu *et al.*, *Virology* 279:78-89 (2001)), were included as a positive control. As a loading control, the same blot was re-probed with a polyclonal antibody against the viral penton base protein.

15 **Preparation of additional Ad5 genomes encoding heterologous fibers**

These same procedures can be used to construct Ad5 genomes containing the 19p (SEQ ID NO. 33), 16 (SEQ ID NO. 37), 30 (SEQ ID NO. 35) and 35 (SEQ ID NO. 39) fibers. To improve incorporation of the fiber in the resulting particle, each fiber was modified to include the N-

20 terminal 61 amino acids of Ad5 (see SEQ ID NO. 2 or see nucleotides 1-183 in SEQ ID NO. 1) by replacing the corresponding amino acids (*i.e.*, the first 61 amino acids) of each heterologous fiber. Similar constructs can be made with other heterologous fibers and genomes, such as Ad2.

For example, for construction of the Ad5/Ad16 chimeric fiber

25 vector, plasmid pDV153 was digested with SphI and MunI to remove all but the first 183 nucleotides of Ad5 fiber. Ad16 fiber (SEQ ID NO. 37) was PCR amplified using 5' primer F16 5' SphI: GCC AGC GGC ATG CTC CAA CTT AAA (SEQ ID NO. 97) and 3' primer F16 3' MunI: TTT ATC AAT TGT GTT GTC AGT CAT CTT C (SEQ ID NO. 98), which contained

-139-

SphI and MunI sites, respectively. The PCR product was ligated with plasmid pDV153 to generate plasmid pDV182. This resulted in expression of a chimeric Ad5/Ad16 fiber protein with the N-terminal 61 amino acids from Ad5 fiber (SEQ ID NO. 2) and the remainder of the
5 protein from Ad16 (corresponding to amino acid 62 to the end of Ad16 fiber; SEQ ID NO. 38).

EXAMPLE 20

Tripartite leader sequences (TPLs) that are useful in enhancing the expression of complementing adenoviral proteins, particularly fiber
10 protein, for use in preparing an adenoviral gene delivery vector are provided. The complete Ad5 TPL was constructed by assembling PCR fragments. First, the third TPL exon (exon 3) (nt 9644-9731 of the Ad5 genome) was amplified from Ad5 genomic DNA using the synthetic oligonucleotide primers 5'CTCAACAATTGT**GGATCC**GTACTCC3' (SEQ ID
15 NO. 99) and 5'GTGCTCAGC**AGATCT**TGCGACTGTG3' (SEQ ID NO. 100). The resulting product was cloned to the BamHI and BglII sites of pΔE1Sp1a (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943) using sites in the primers (shown in bold) to create plasmid pDV52. A fragment corresponding to the first TPL exon
20 (exon 1), the natural first intron (intron 1), and the second TPL exon (exon 2) (Ad5 nt 6049-7182) was then amplified using primers 5'GGCGCGTT**CGGATCC**ACTCTCTTCC3' (SEQ ID NO. 101) and 5'CTACATGCTAGGC**AGATCT**CGTTCCGGAG3' (SEQ ID NO. 102), and cloned into the BamHI site of pDV52 (again using sites in the primers) to
25 create pDV55.

This plasmid contains a 1.2 kb BamHI/BglII fragment containing the first TPL exon, the natural first intron, and the fused second and third TPL exons. The nucleotide sequence of the complete TPL containing the noted 5' and 3' restriction sites is shown in SEQ ID NO. 103 with the

-140-

following nucleotide regions identified: 1-6 nt BamHI site; 7-47 nt first leader segment (exon 1); 48-1068 nt natural first intron (intron 1); 1069-1140 nt second leader segment (exon 2); 1141-1146 nt fused BamHI and BglII sites; 1147-1234 nt third leader segment (exon 3); and 1235-1240 nt BglII site.

EXAMPLE 21

Preparation of Adenoviral Gene Delivery Vectors Using Adenoviral Packaging Cell Lines

Adenoviral delivery vectors are prepared to separately lack the combinations of E1/fiber and E4/fiber. Such vectors are more replication-defective than those previously in use due to the absence of multiple viral genes. A preferred adenoviral delivery vector is replication competent but only via a non-fiber means is one that only lacks the fiber gene but contains the remaining functional adenoviral regulatory and structural genes. Furthermore, these adenovirus delivery vectors have a higher capacity for insertion of foreign DNA.

A. Preparation of Adenoviral Gene Delivery Vectors Having Specific Gene Deletions and Methods of Use

To construct an E1/fiber deleted viral vector containing the LacZ reporter gene construct, two new plasmids were constructed. The plasmid p Δ E1B β gal was constructed as follows. A DNA fragment containing the SV40 regulatory sequences and *E. coli* β -galactosidase gene was isolated from pSV β gal (Promega) by digesting with VspI, filling the overhanging ends by treatment with Klenow fragment of DNA polymerase I in the presence of dNTPs and digesting with BamHI. The resulting fragment was cloned into the EcoRV and BamHI sites in the polylinker of p Δ E1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087 and U.S. Patent No. 6,379,943) to form p Δ E1B β gal that therefore contained the left end of the adenovirus genome with the Ela region replaced by the LacZ cassette (nucleotides 6690 to 4151) of

-141-

pSV β gal. Plasmid DNA may be prepared by the alkaline lysis method as described by Birnboim and Doly, *Nuc. Acids Res.*, 7:1513-1523 (1978) or by the Quiagen method according to the manufacturer's instruction, from transformed cells used to expand the plasmid DNA. Plasmid DNA was
5 then purified by CsCl-ethidium bromide density gradient centrifugation. Alternatively, plasmid DNAs may be purified from *E. coli* by standard methods known in the art (e.g. see Sambrook *et al.*)

The second plasmid (pDV44), prepared as described herein, is derived from pBHG10, a vector prepared as described by Bett *et al.*, *Proc.*
10 *Natl. Acad. Sci., USA*, 91:8802-8806 (1994) (see, also International PCT application No. WO 95/00655) using methods well known to one of skill in the art. This vector also is commercially available from Microbix Biosystems and contains an Ad5 genome with the packaging signals at the left end deleted and the E3 region (nucleotides 28133:30818)
15 replaced by a linker with a unique site for the restriction enzyme PacI. An 11.9 kb BamHI fragment, which contains the right end of the adenovirus genome, is isolated from pBHG10 and cloned into the BamHI site of pBS/SK(+) to create plasmid p11.3 having approximately 14,658 bp. The p11.3 plasmid was then digested with PacI and Sall to remove the
20 fiber, E4, and inverted terminal repeat (ITR) sequences.

This fragment was replaced with a 3.4 kb fragment containing the ITR segments and the E4 gene which was generated by PCR amplification from pBHG10 using the following oligonucleotide sequences:
5' TGTACACCG GATCCGGCGCACACC3' SEQ ID NO: 104; and
25 5' CACAACGAGCTC AATTAATTAATTGCCACATCCTC3' SEQ ID NO: 105. These primers incorporated sites for PacI and BamHI. Cloning this fragment into the PacI and blunt ended Sall sites of the p11.3 backbone resulted in a substitution of the fused ITRs, E4 region and fiber gene present in pBHG10, by the ITRs and E4 region alone. The resulting

-142-

p11.3 plasmid containing the ITR and E4 regions, designated plasmid pDV43a, was then digested with BamHI. This BamHI fragment was then used to replace a BamHI fragment in pBHG10 thereby creating pDV44 in a pBHG10 backbone.

- 5 In an alternative approach to preparing pDV44 with an additional subcloning step to facilitate the incorporation of restriction cloning sites, the following cloning procedure was performed. pDV44 as above was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10 (Microbix Biosystems; see, also U.S. Patent No.
- 10 6,140,087). As above, to simplify manipulations, the 11.9 kb BamHI fragment including the rightmost part of the Ad5 genome was removed from pBHG10 and inserted into pBS/SK. The resulting plasmid was termed p11.3. The 3.4 kb DNA fragment corresponding to the E4 region and both ITRs of adenovirus type 5 was amplified as described above
- 15 from pBHG10 using the oligonucleotides listed above and subcloned into the vector pCR2.1 (Invitrogen) to create pDV42. This step is the additional cloning step to facilitate the incorporation of a Sall restriction site. pDV42 was then digested with PacI, which cuts at a unique site (bold type) in one of the PCR primers, and with Sall, which cuts at a
- 20 unique site in the pCR2.1 polylinker. This fragment was used to replace the corresponding PacI/XhoI fragment of p11.3 (the pBS polylinker adjacent to the Ad DNA fragment contains a unique XhoI site), creating pDV43. A plasmid designated pDV44 was constructed by replacing the 11.9 kb BamHI fragment of pBHG10 by the analogous BamHI fragment of
- 25 pDV43. As generated in the first procedure, pDV44 therefore differs from pBHG10 by the deletion of Ad5 nucleotides 30819:32743 (residual E3 sequences and all but the 3'-most 41 nucleotides of the fiber open reading frame).

-143-

In summary, the cloning procedures described above result in the production of a fiber-deleted Ad5 genomic plasmid (pDV44) that was constructed by removing the fiber gene and some of the residual E3 sequences from pBHG10. pDV44 contains a wild-type E4 region, but
5 only the last 41 nucleotides of the fiber ORF (this sequence was retained to avoid affecting expression of the adjacent E4 transcription unit). Plasmids pBHG10 and pDV44 contain unpackageable Ad5 genomes, and must be rescued by cotransfection and subsequent homologous recombination with DNA carrying functional packaging signals. In order
10 to generate vectors marked with a reporter gene, either pDV44 or pBHG10 was cotransfected with p Δ E1B β gal, which contains the left end of the Ad5 genome with an SV40-driven β -galactosidase reporter gene inserted in place of the E1 region.

In general, and as described below, the method for virus production
15 by recombination of plasmids followed by complementation in cell culture involves the isolation of recombinant viruses by cotransfection of any adenovirus packaging cell system, namely 211A, 211B, 211R, A549, Vero cells, and the like, with plasmids carrying sequences corresponding to viral gene delivery vectors.

20 A selected cell line is plated in dishes and cotransfected with pDV44 and p Δ E1B β gal using the calcium phosphate method as described by Bett *et al.*, *Proc. Natl. Acad. Sci., USA*, 91:8802-8806 (1994). Recombination between the overlapping adenovirus sequences in the two plasmids leads to the creation of a full-length viral chromosome where
25 pDV44 and p Δ E1B β gal recombine to form a recombinant adenovirus vector having multiple deletions. The deletion of E1 and of the fiber gene from the viral chromosome is compensated for by the sequences integrated into the packaging cell genome, and infectious virus particles

-144-

are produced. The plaques thus generated are isolated and stocks of the recombinant virus are produced by standard methods.

Because of the fiber deletion, a pDV44-derived virus is replication-defective, and cells in which it is grown must complement this defect.

- 5 The 211B cell line (a derivative of 293 cells which expresses the wild-type (wt) Ad5 fiber and is equivalent to 211A on deposit with ATCC) was used for rescue and propagation of the virus described here. pDV44 and p Δ E1 β gal were cotransfected into 211B cells, and the monolayers were observed for evidence of cytopathic effect (CPE). Briefly, for virus
- 10 construction, cells were transfected with the indicated plasmids using the Gibco Calcium Phosphate Transfection system according to the manufacturer's instructions and observed daily for evidence of CPE.

- One of a total of 58 transfected dishes showed evidence of spreading cell death at day 15. A crude freeze-thaw lysate was prepared
- 15 from these cells and the resulting virus (termed Ad5. β gal. Δ F) was plaque purified twice and then expanded. To prepare purified viral preparations, cells were infected with the indicated Ad and observed for completion of CPE. Briefly, at day zero, 211B cells were plated in DMEM plus 10% fetal calf serum at approximately 1×10^7 cells/150 cm² flask or
- 20 equivalent density. At day one, the medium was replaced with one half the original volume of fresh DMEM containing the indicated Ad, in this case Ad5. β gal. Δ F, at approximately 100 particles/cell. At day two, an equal volume of medium was added to each flask and the cells were observed for CPE. Two to five days after infection, cells were collected
- 25 and virus isolated by lysis via four rapid freeze-thaw cycles. Virus was then purified by centrifugation on preformed 15-40% CsCl gradients (111,000 x g for three hours at 4°C). The bands were harvested, dialyzed into storage buffer (10 mM Tris-pH 8.1, 0.9% NaCl, and 10% glycerol), aliquoted and stored at - 70°C. Purified Ad5. β gal. Δ F virus

-145-

particles containing human adenovirus Ad5. β gal. Δ F genome (described further below) have been deposited with the ATCC on January 15, 1999.

For viral titering, Ad preparations were titered by plaque assay on 211B cells. Cells were plated on polylysine-coated 6 well plates at 1.5×10^6 cells/well. Duplicate dilutions of virus stock were added to the plates in 1 ml/well of complete DMEM. After a five hour incubation at 37°C, virus was removed and the wells overlaid with 2 ml of 0.6% low-melting agarose in Medium 199 (Gibco). An additional 1 ml of overlay was added at five day intervals.

As a control, the first-generation virus Ad5. β gal.wt, which is identical to Ad5. β gal. Δ F except for the fiber deletion, was constructed by cotransfection of pBHG10 and p Δ E1B β gal. In contrast to the low efficiency of recovery of the fiberless genome (1/58 dishes), all of 9 dishes cotransfected with p Δ E1B β gal and pBHG10 produced virus.

In another embodiment, a delivery plasmid is prepared that does not require the above-described recombination events to prepare a viral vector having a fiber gene deletion. In one embodiment, a single delivery plasmid containing all the adenoviral genome necessary for packaging but lacking the fiber gene is prepared from plasmid pFG140 containing full-length Ad5 that is commercially available from Microbix. The resultant delivery plasmid referred to as pFG140-f is then used with pCLF (ATCC accession number 97737; and described in copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000)) stably integrated cells as described above to prepare a viral vector lacking fiber. For genetic therapy, the fiber gene can be replaced with a therapeutic gene of interest for preparing a therapeutic delivery adenoviral vector.

Vectors for the delivery of any desired gene and preferably a therapeutic gene are prepared by cloning the gene of interest into the

-146-

multiple cloning sites in the polylinker of commercially available p Δ E1sp1B (Microbix Biosystems; see also, U.S. Patent No. 6,140,087), in an analogous manner as performed for preparing pE1B β gal as described above. The same cotransfection and recombination procedure is then followed as described herein to obtain viral gene delivery vectors.

1. Characterization of the Ad5. β gal. Δ F Genome

To confirm that the vector genomes had the proper structures and that the fiber gene was absent from the Ad5. β gal. Δ F chromosome, the DNA isolated from viral particles was analyzed. Briefly, purified viral DNA was obtained by adding 10 μ l of 10 mg/ml proteinase K, 40 μ l of 0.5 M EDTA and 50 μ l of 10% SDS to 800 μ l of adenovirus-containing culture supernatant. The suspension was then incubated at 55°C for 60 minutes. The solution was then extracted once with 400 μ l of a 24:1 mixture of chloroform:isoamyl alcohol. The aqueous phase was then removed and precipitated with sodium acetate/ethanol. The pellet was washed once with 70% ethanol and lightly dried. The pellet was then suspended in 40 μ l of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Genomic DNA from Ad5. β gal.wt and Ad5. β gal. Δ F produced the expected restriction patterns following digestion with either EcoRI or with NdeI. Southern blotting, performed with standard methods, with labeled fiber DNA as a probe demonstrated the presence of fiber sequence in Ad5. β gal.wt but not in Ad5. β gal. Δ F DNA. As a positive control, the blot was stripped and reprobed with labeled E4 sequence. Fiber and E4 sequences were detected by using labeled inserts from pCLF and pE4/Hygro, respectively. E4 signal was readily detectable in both genomes at equal intensities. The complete nucleotide sequence of Ad5. β gal. Δ F is presented in SEQ ID NO: 106 and is contained in the virus particle deposited with ATCC.

-147-

2. Characterization of the Fiberless Adenovirus Ad5. β gal. Δ F

To verify that Ad5. β gal. Δ F was fiber-defective, 293 cells (which are permissive for growth of E1-deleted Ad vectors but do not express fiber) were infected with Ad5. β gal. Δ F or with Ad5. β gal.wt. Twenty-four hours post infection, the cells were stained with polyclonal antibodies directed either against fiber or against the penton base protein. Cells infected with either virus were stained by the anti-penton base antibody, while only cells infected with the Ad5. β gal.wt control virus reacted with the anti-fiber antibody. This confirms that the fiber-deleted Ad mutant does not direct the synthesis of fiber protein.

3. Growth of the Fiber-Deleted Ad5. β gal. Δ F Vector in Complementing Cells

Ad5. β gal. Δ F was found to readily be propagated in 211B cells. As assayed by protein concentration, CsCl-purified stocks of either Ad5. β gal. Δ F or Ad5. β gal.wt contained similar numbers of viral particles. The particles appeared to band normally on CsCl gradients. Infectivity of the Ad5. β gal. Δ F particles was lower than the Ad5. β gal.wt control, as indicated by an increased particle/PFU ratio. Ad5. β gal. Δ F was also found to plaque more slowly than the control virus. When plated on 211B cells, Ad5. β gal.wt plaques appeared within 5-7 days, while plaques of Ad5. β gal. Δ F continued to appear until as much as 15-18 days post infection. Despite their slower formation, the morphology of Ad5. β gal. Δ F plaques was essentially normal.

4. Production of Fiberless Ad5. β gal. Δ F Particles

As Ad5. β gal. Δ F represents a true fiber null mutation and its stocks are free of helper virus, the fiber mutant phenotype was readily investigated. A single round of growth in cells (such as 293) which do not produce fiber generating a homogeneous preparation of fiberless Ad allowed for the determination of whether such particles would be stable

-148-

and/or infectious. Either Ad5. β gal.wt or Ad5. β gal. Δ F was grown in 293 or 211B cells, and the resulting particles purified on CsCl gradients as previously described. Ad5. β gal. Δ F particles were readily produced in 293 cells at approximately the same level as the control virus and behaved
5 similarly on the gradients, indicating that there was not a gross defect in morphogenesis of fiberless capsids.

Particles of either virus contained similar amounts of penton base regardless of the cell type in which they were grown. This demonstrated that fiber is not required for assembly of the penton base complex into
10 virions. The Ad5. β gal. Δ F particles produced in 293 cells did not contain fiber protein. 211B-grown Ad5. β gal. Δ F also contained less fiber than the Ad5. β gal.wt control virus. The infectivities of the different viral preparations on epithelial cells correlated with the amount of fiber protein present. The fiberless Ad particles were several thousand-fold less
15 infectious than the first-generation vector control on a per-particle basis, while infectivity of 211B-grown Ad5. β gal. Δ F was only 50-100 fold less than that of Ad5. β gal.wt. These studies confirmed fiber's crucial role in infection of epithelial cells via CAR binding.

20 5. **Composition and Structure of the Fiberless Ad5. β gal. Δ F Particles**

The proteins contained in particles of 293-grown Ad5. β gal. Δ F were compared to those in Ad5. β gal.wt, to determine whether proteolysis or particle assembly was defective in this fiber null mutant. The overall pattern of proteins in the fiberless particles was observed to be quite
25 similar to that of a first-generation vector, with the exception of reduced intensity of the composite band resulting from proteins IIIa and IV (fiber). The fiberless particles also had a reduced level of protein VII. Although substantial amounts of uncleaved precursors to proteins VI, VII, and VIII were not seen, it is possible that the low-molecular weight bands

-149-

migrating ahead of protein VII represent either aberrantly cleaved viral proteins or their breakdown products.

Cryo-electron microscopy was used to more closely examine the structure of the 293 grown Ad5.βgal.ΔF and of Ad5βgal.wt. The fiber, having an extended stalk with a knob at the end, was faintly visible in favorable orientations of wild-type Ad5 particles, but not in images of the fiberless particles. Filamentous material likely corresponding to free viral DNA was seen in micrographs of fiberless particles. This material was also present in micrographs of the first-generation control virus, albeit at much lower levels.

Three-dimensional image reconstructions of fiberless and wild-type particles at ~20 Å resolution showed similar sizes and overall features, with the exception that fiberless particles lacked density corresponding to the fiber protein. The densities corresponding to other capsid proteins, including penton base and proteins IIIa, VI, and IX, were comparable in the two structures. This confirms that absence of fiber does not prevent assembly of these components into virions. The fiber was truncated in the wild-type structure as only the lower portion of its flexible shaft follows icosahedral symmetry. The RGD protrusions on the fiberless penton base were angled slightly inward relative to those of the wild-type structure. Another difference between the two penton base proteins was that there is a ~30 Å diameter depression in the fiberless penton base around the five-fold axis where the fiber would normally sit. The Ad5 reconstructions confirm that capsid assembly, including addition of penton base to the vertices, is able to proceed in the complete absence of fiber.

-150-

6. Integrin-Dependent Infectivity of Fiberless Ad5. β gal. Δ F Particles

While attachment via the viral fiber protein is a critical step in the infection of epithelial cells, an alternative pathway for infection of certain hematopoietic cells has been described. In this case, penton base mediates binding to the cells (via β 2 integrins) and internalization (through interaction with α v integrins). Particles lacking fiber might therefore be expected to be competent for infection of these cells, even though on a per-particle basis they are several thousand-fold less infectious than normal Ad vectors on epithelial cells.

To investigate this, THP-1 monocytic cells were infected with Ad5. β gal.wt or with Ad5. β gal. Δ F grown in the absence of fiber. Infection of THP-1 cells was assayed by infecting 2×10^5 cells at the indicated m.o.i. in 0.5 ml of complete RPMI. Forty-eight hours post-infection, the cells were fixed with glutaraldehyde and stained with X-gal, and the percentage of stained cells was determined by light microscopy. The results of the infection assay showed that the fiberless particles were only a few-fold less infectious than first-generation Ad on THP-1 cells. Large differences were seen in plaquing efficiency on epithelial (211B) cells. Infection of THP-1 cells by either Ad5. β gal. Δ F or Ad5. β gal.wt was not blocked by an excess of soluble recombinant fiber protein, but could be inhibited by the addition of recombinant penton base). These results indicate that the fiberless Ad particles use a fiber-independent pathway to infect these cells. Furthermore, the lack of fiber protein did not prevent Ad5. β gal. Δ F from internalizing into the cells and delivering its genome to the nucleus, demonstrating that fiberless particles are properly assembled and are capable of uncoating.

The foregoing results with the recombinant viruses thus produced indicates that they can be used as gene delivery tools in cultured cells

-151-

and *in vivo*. For example, for studies of the effectiveness and relative immunogenicity of multiply-deleted vectors, virus particles are produced by growth in packaging lines and are purified by CsCl gradient centrifugation. Following titering, virus particles are administered to mice
5 via systemic or local injection or by aerosol delivery to lung. The LacZ reporter gene allows the number and type of cells which are successfully transduced to be evaluated. The duration of transgene expression is evaluated in order to determine the long-term effectiveness of treatment with multiply-deleted recombinant adenoviruses relative to the standard
10 technologies which have been used in clinical trials to date. The immune response to the improved vectors described here is determined by assessing parameters such as inflammation, production of cytotoxic T lymphocytes directed against the vector, and the nature and magnitude of the antibody response directed against viral proteins.

15 Versions of the vectors which contain therapeutic genes such as CFTR for treatment of cystic fibrosis or tumor suppressor genes for cancer treatment are evaluated in the animal system for safety and efficiency of gene transfer and expression. Following this evaluation, they are used as experimental therapeutic agents in human clinical trials.

20 **B. Retargeting of Adenoviral Gene Delivery Vectors by Producing Viral Particles Containing Different or Altered Fiber Proteins**

As the specificity of adenovirus binding to target cells is largely determined by the fiber protein, viral particles that incorporate modified
25 fiber proteins or fiber proteins from different adenoviral serotypes (pseudotyped vectors) have different specificities. Thus, the methods of expression of the native Ad5 fiber protein in adenovirus packaging cells as described above also is applicable to production of different fiber proteins.

-152-

Chimeric fiber proteins can be produced according to known methods (see, *e.g.*, Stevenson *et al.* (1995) *J. Virol.*, 69:2850-2857). Determinants for fiber receptor binding activity are located in the head domain of the fiber and an isolated head domain is capable of

5 trimerization and binding to cellular receptors. The head domains of adenovirus type 3 (Ad3) and Ad5 were exchanged in order to produce chimeric fiber proteins. Similar constructs for encoding chimeric fiber proteins for use in the methods herein are contemplated. Thus, instead of using the intact Ad5 fiber-encoding construct (prepared above and in U.S. application Serial No. 09/482,682) as a complementing viral vector in
10 adenoviral packaging cells, the constructs described herein are used to transfect cells along with E4 and/or E1-encoding constructs.

Briefly, full-length Ad5 and Ad3 fiber genes were amplified from purified adenovirus genomic DNA as a template. The Ad5 and Ad3
15 nucleotide sequences are available with the respective GenBank Accession Numbers M18369 and M12411. Oligonucleotide primers are designed to amplify the entire coding sequence of the full-length fiber genes, starting from the start codon, ATG, and ending with the termination codon TAA. For cloning purposes, the 5' and 3' primers
20 contain the respective restriction sites BamHI and NotI for cloning into pcDNA plasmid. PCR is performed as described above.

The resulting products are then used to construct chimeric fiber constructs by PCR gene overlap extension (Horton *et al.* (1990) *BioTechniques*, 8:525-535). The Ad5 fiber tail and shaft regions (5TS;
25 the nucleotide region encoding amino acid residue positions 1 to 403) are connected to the Ad3 fiber head region (3H; the nucleotide region encoding amino acid residue positions 136 to 319) to form the 5TS3H fiber chimera. Conversely, the Ad3 fiber tail and shaft regions (3TS; the nucleotide region encoding amino acid residues positions 1 to 135) are

-153-

connected to the Ad5 fiber head region (5H; the nucleotide region encoding the amino acid residue positions 404 to 581) to form the 3TS5H fiber chimera. The fusions are made at the conserved TLWT (SEQ ID NO: 46) sequence at the fiber shaft-head junction.

5 The resultant chimeric fiber PCR products are then digested with BamHI and NotI for separate directional ligation into a similarly digested pcDNA3.1. The TPL sequence is then subcloned into the BamHI for preparing an expression vector for subsequent transfection into 211 cells or into alternative packaging cell systems. The resultant chimeric fiber
10 construct-containing adenoviral packaging cell lines are then used to complement adenoviral delivery vectors as previously described. Other fiber chimeric constructs are obtained with the various adenovirus serotypes using a similar approach.

 In an alternative embodiment, the use of modified proteins
15 including with modified epitopes (see, *e.g.*, Michael *et al.* (1995) *Gene Therapy*, 2:660-668 and International PCT application Publication No. WO 95/26412, which describe the construction of a cell-type specific therapeutic viral vector having a new binding specificity incorporated into the virus concurrent with the destruction of the endogenous viral binding
20 specificity). In particular, the authors described the production of an adenoviral vector encoding a gastrin releasing peptide (GRP) at the 3' end of the coding sequence of the Ad5 fiber gene. The resulting fiber-GRP fusion protein was expressed and shown to assemble functional fiber trimers that were correctly transported to the nucleus of HeLa cells
25 following synthesis.

 Similar constructs are contemplated for use in the complementing adenoviral packaging cell systems for generating new adenoviral gene delivery vectors that are targetable, replication-deficient and less immunogenic. Heterologous ligands contemplated for use herein to

-154-

redirect fiber specificity range from as few as 10 amino acids in size to large globular structures, some of which necessitate the addition of a spacer region so as to reduce or preclude steric hindrance of the heterologous ligand with the fiber or prevent trimerization of the fiber protein. The ligands are inserted at the end or within the linker region. Preferred ligands include those that target specific cell receptors or those that are used for coupling to other moieties such as biotin and avidin.

A preferred spacer includes a short 12 amino acid peptide linker composed of a series of serines and alanine flanked by a proline residue at each end using routine procedures known to those of skill in the art. The skilled artisan will be with the preparation of linkers to accomplish sufficient protein presentation and to alter the binding specificity of the fiber protein without compromising the cellular events that follow viral internalization. Moreover, within the context of this disclosure, preparation of modified fibers having ligands positioned internally within the fiber protein and at the carboxy terminus as described below are contemplated for use with the methods described herein.

The preparation of a fiber having a heterologous binding ligand is prepared essentially as described in the above-cited paper. Briefly, for the ligand of choice, site-directed mutagenesis is used to insert the coding sequence for a linker into the 3' end of the Ad5 fiber construct in pCLF.

The 3' or antisense or mutagenic oligonucleotide encodes a preferred linker sequence of ProSerAlaSerAlaSerAlaSerAlaProGlySer (SEQ ID NO: 107) followed by a unique restriction site and two stop codons, respectively, to allow the insertion of a coding sequence for a selected heterologous ligand and to ensure proper translation termination. Flanking this linker sequence, the mutagenic oligonucleotide contains sequences that overlap with the vector sequence and allow its incorporation into the construct. Following mutagenesis of the pCLF sequence adding the

-155-

linker and stop codon sequences, a nucleotide sequence encoding a preselected ligand is obtained, linkers corresponding to the unique restriction site in the modified construct are attached and then the sequence is cloned into linearized corresponding restriction site.

- 5 The resultant fiber-ligand construct is then used to transfect 211 or the alternative cell packaging systems previously described to produce complementing viral vector packaging systems.

In a further embodiment, intact fiber genes from different Ad serotypes are expressed by 211 cells or an alternative packaging system.

- 10 A gene encoding the fiber protein of interest is first cloned to create a plasmid analogous to pCLF, and stable cell lines producing the fiber protein are generated as described above for Ad5 fiber. The adenovirus vector described which lacks the fiber gene is then propagated in the cell line producing the fiber protein relevant for the purpose at hand. As the
- 15 only fiber gene present is the one in the packaging cells, the adenoviruses produced contain only the fiber protein of interest and therefore have the binding specificity conferred by the complementing protein. Such viral particles are used in studies such as those described above to determine their properties in experimental animal systems.

20

EXAMPLE 22

Preparation and Use of Adenoviral Packaging Cell Lines Containing Plasmids Containing Alternative TPLs

- Plasmids containing tripartite leaders (TPLs) have been constructed. The resulting plasmids that contain different selectable markers, such as
- 25 neomycin and zeocin, were then used to prepare fiber-complementing stable cell lines for use as for preparing adenoviral vectors.

A. pDV60

Plasmid pDV60 was constructed by inserting the TPL cassette of SEQ ID NO. 88 into the BamHI site upstream of the Ad5 fiber gene in

-156-

pcDNA3/Fiber, a neomycin selectable plasmid (see, *e.g.*, U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000); see also Von Seggern *et al.* (1998) *J. Gen Virol.*, 79: 1461-1468). The nucleotide sequence of
5 pDV60 is listed in SEQ ID NO: 108. Plasmid pDV60 is available from the ATCC under accession number PTA-1144.

B. pDV61

To construct pDV61, an Asp718/NotI fragment containing the CMV promoter, partial Ad5 TPL, wildtype Ad5 fiber gene, and bovine
10 growth hormone terminator was transferred from pCLF (ATCC accession number 97737; and described in copending U.S. application Serial No. 09/482,682 (also filed as International PCT application No. PCT/US00/00265 on January 14, 2000)), to a zeocin selectable cloning
vector referred to as pCDNA3.1/Zeo (+) (commercially available from
15 Invitrogen and for which the sequence is known).

C. pDV67

In an analogous process, pDV67 containing complete TPL was constructed by transferring an Asp 718/XbaI fragment from pDV60 into
pcDNA3.1/Zeo(+) backbone. The nucleotide sequence of pDV67 is set
20 forth in SEQ ID NO. 109. Plasmid pDV67 is available from the ATCC under accession number PTA-1145.

D. pDV69

To prepare pDV69 containing a modified fiber protein, the chimeric Ad3/Ad5 fiber gene was amplified from pGEM5TS3H (Stevenson *et al.*
25 (1995) *J. Virol.*, 69: 2850-2857) using the primers 5'ATGGGAT CAAGATGAAGCGCGCAAGACCG3' (SEQ ID NO. 110) and 5'CACTATAGCGGCCGCATTCTCAGTCATCTT3' (SEQ ID NO. 111), and cloned to the BamHI and NotI sites of pcDNA3.1/Zeo(+) via new BamHI and NotI sites engineered into the primers to create pDV68. Finally, the

-157-

complete TPL fragment described above was then added to the unique BamHI site of pDV68 to create pDV69. The nucleotide sequence of pDV69 is listed in SEQ ID NO. 112 and the plasmid is available from the ATCC under accession number PTA-1146.

5 E. Preparation of Stable Adenovirus Packaging Cell Lines

E1-2a S8 cells are derivatives of the A549 lung carcinoma line (ATCC # CCL 185) with chromosomal insertions of the plasmids pGRE5-2.E1 (also referred to as GRE5-E1-SV40-Hygro construct and listed in SEQ ID NO. 47) and pMNeoE2a-3.1 (also referred to as MMTV-
10 E2a-SV40-Neo construct and listed in SEQ ID NO. 48), which provide complementation of the adenoviral E1 and E2a functions, respectively. This line and its derivatives were grown in Richter's modified medium (BioWhitaker) + 10% FCS. E1-2a S8 cells were electroporated as previously described (Von Seggern *et al.* (1998) *J. Gen Virol.*, 79: 1461-
15 1468) with pDV61, pDV67, or with pDV69, and stable lines were selected with zeocin (600 μ g/ml).

The cell line generated with pDV61 is designated 601. The cell line generated with pDV67 is designated 633 while that generated with pDV69 is designated 644. Candidate clones were evaluated by
20 immunofluorescent staining with a polyclonal antibody raised against the Ad2 fiber. Lines expressing the highest level of fiber protein were further characterized.

For the S8 cell complementing cell lines, to induce E1 expression, 0.3 μ M of dexamethasone was added to cell cultures 16-24 hours prior to
25 challenge with virus for optimal growth kinetics. For preparing viral plaques, 5×10^5 cells/well in 6 well plates are prepared and pre-induced with the same concentration of dexamethasone the day prior to infection with 0.5 μ M included at a final concentration in the agar overlay after infection.

-158-

F. Cell Lines for Complementation of E1⁻/E2a⁻ Vectors

The Adenovirus 5 genome was digested with Scal enzyme, separated on an agarose gel, and the 6,095 bp fragment containing the left end of the virus genome was isolated. The complete Adenovirus 5 genome is registered as Genbank accession #M73260 (or see SEQ ID NO. 1), incorporated herein by reference, and the virus is available from the American Type Culture Collection, Manassas, Virginia, U.S.A., under accession number VR-5. The Scal 6,095 bp fragment was digested further with Clal at bp 917 and BglII at bp 3,328. The resulting 2,411 bp Clal to BglII fragment was purified from an agarose gel and ligated into the superlinker shuttle plasmid pSE280 (Invitrogen, San Diego, CA), which was digested with Clal and BglII, to form pSE280-E.

Polymerase chain reaction (PCR) was performed to synthesize DNA encoding an XhoI and SalI restriction site contiguous with Adenovirus 5 DNA bp 552 through 924. The primers which were employed were as follows:

5' end, Ad5 bp 552-585:

5'-GTCACTCGAGGACTCGGTC-GACTGAAAATGAGACATATTATCTGCC
ACGGACC-3' (SEQ ID NO. 113)

20 3' end, Ad5 bp 922-891:

5'-CGAGATCGATCACCTCCGGTACAAGGTTTGGCATAG-3' (SEQ ID NO.
114)

This amplified DNA fragment (also referred to herein as Fragment A) was digested with XhoI and Clal, which cleaves at the native Clal site (bp 917), and ligated to the XhoI and Clal sites of pSE280-E, thus reconstituting the 5' end of the E1 region beginning 8 bp upstream of the ATG codon. PCR amplification then was performed to amplify Ad 5 DNA from bp 3,323 through 4,090 contiguous with an EcoRI restriction site. The primers employed were as follows:

-159-

5' end, Ad5 bp 3323-3360:

5'-CATGAAGATCTGGAAGGTGCTGAGGTACGATGAGACC-3' (SEQ ID NO. 115); and

3' end, Ad5 bp 4090-4060:

5 5'-GCGACTTAAGCAGTCAGCTG-AGACAGCAAGACACTTGCTTGATCCA AATCC-3' (SEQ ID NO. 116).

This amplified DNA fragment (also referred to herein as Fragment B) was digested with BglII, thereby cutting at the Adenovirus 5 BglII site (bp 3,382) and EcoRI, and ligated to the BglII and EcoRI sites of
10 pSE280-AE to reconstruct the complete E1a and E1b region from Adenovirus 5 bp 552 through 4,090. The resulting plasmid is designated pSE280-E1.

A construct containing the intact E1a/b region under the control of the synthetic promoter GRE5 was prepared as follows. The intact E1a/b
15 region was excised from pSE280-E1, which was modified previously to contain a BamHI site 3' to the E1 gene, by digesting with XhoI and BamHI. The XhoI to BamHI fragment containing the E1a/b fragment was cloned into the unique XhoI and BamHI sites of pGRE5-2/EBV (U.S. Biochemicals, Cleveland, Ohio) to form pGRE5-E1).

20 Bacterial transformants containing the final construct were identified. Plasmid DNA was prepared and purified by banding in CsTFA prior to use for transfection of cells.

G. Construction of plasmid including Adenovirus 5 E2A sequence

25 The Adenovirus 5 genome was digested with BamHI and SpeI, which cut at bp 21,562 and 27,080, respectively. Fragments were separated on an agarose gel and the 5,518 bp BamHI to SpeI fragment was isolated. The 5,518 bp BamHI to SpeI fragment was digested further with SmaI, which cuts at bp 23,912. The resulting 2,350 bp BamHI to

-160-

SmaI fragment was purified from an agarose gel, and ligated into the superlinker shuttle plasmid pSE280, and digested with BamHI and SmaI to form pSE280-E2 BamHI-SmaI.

PCR then was performed to amplify Adenovirus 5 DNA from the
5 SmaI site at bp 23,912 through 24,730 contiguous with NheI and EcoRI restriction sites. The primers which were employed were as follows:

5' end, Ad5 bp 24,732-24,708:

5'-CACGAATTCGTCAGCGCTTCTCGTCGCGTCCAAGACCC-3' (SEQ ID NO. 117);

10 3' end, Ad5 bp 23,912-23,934:

5'-CACCCCGGGGAGGCGGCGGCGACGGGGACGGG-3' (SEQ ID NO. 118)

This amplified DNA fragment was digested with SmaI and EcoRI, and ligated to the SmaI and EcoRI sites of pSE280-E2 Bam-SmaI to reconstruct the complete E2a region from Ad5 bp 24,730 through
15 21,562. The resulting construct is pSE280-E2a.

In order to convert the BamHI site at the 3' end of E2a to a Sall site, the E2a region was excised from pSE280-E2a by cutting with BamHI and NheI, and recloned into the unique BamHI and NheI sites of pSE280. Subsequently, the E2a region was excised from this construction with
20 NheI and Sall in order to clone into the NheI and Sall sites of the pMAMneo (Clonotech, Palo Alto, CA) multiple cloning site in a 5' to 3' orientation, respectively. The resulting construct is pMAMneo E2a.

Bacterial transformants containing the final pMAMneo-E2a were identified. Plasmid DNA was prepared and purified by banding in CsTFA.
25 Circular plasmid DNA was linearized at the XmnI site within the ampicillin resistance gene of pMAMneo-E2a, and further purified by the phenol/chloroform extraction and ethanol precipitation prior to use for transfection of cells.

-161-

H. Transfection and selection of cells

In general, this process involved the sequential introduction, by calcium phosphate precipitation, or other means of DNA delivery, of two plasmid constructions each with a different viral gene, into a single tissue culture cell. The cells were transfected with a first construct and selected for expression of the associated drug resistance gene to establish stable integrants. Individual cell clones were established and assayed for function of the introduced viral gene. Appropriate candidate clones then were transfected with a second construct including a second viral gene and a second selectable marker. Transfected cells then were selected to establish stable integrants of the second construct, and cell clones were established. Cell clones were assayed for functional expression of both viral genes.

A549 (ATCC Accession No. CCL-185) were used for transfection. Appropriate selection conditions were established for G418 and hygromycin B by standard kill curve determination.

Transfection of A549 cells with plasmids including E1 and E2a regions.

pMAMNeo-E2a was linearized with XmnI with the Amp^R gene, introduced into cells by transfection, and cells were selected for stable integration of this plasmid by G418 selection until drug resistant colonies arose. The clones were isolated and screened for E2a expression by staining for E2a protein with a polyclonal antiserum, and visualizing by immunofluorescence. E2a function was screened by complementation of the temperature-sensitive mutant Ad5ts125 virus which contains a temperature-sensitive mutation in the E2a gene. (Van Der Vliet, et al., J. Virology, Vol. 15, pgs. 348-354 (1975)). Positive clones expressing the E2a gene were identified and used for transfection with the 7 kb EcoRV to XmnI fragment from pGRE5-E1, which contains the GRE5 promoted

-162-

E1a/b region plus the hygromycin^R gene. Cells were selected for hygromycin resistance and assayed for E1a/b expression by staining with a monoclonal antibody for the E1 protein (Oncogene Sciences, Uniondale, N.Y.). E1 function was assayed by ability to complement an E1-deleted
5 vector. At this point, expression and function of E2a was verified as described above, thus establishing the expression of E1a/b and E2a in the positive cell clones.

A transfected A549 (A549 (ATCC Accession No. CCL-185);) cell line showed good E1a/b and E2a expression and was selected for further
10 characterization. It was designated the S8 cell line.

I. Preparation of Adenoviral Vectors Containing Ad5. β gal. Δ F Genome in S8 Fiber-Complementing Cell Lines

To prepare adenoviral vectors containing Ad5. β gal. Δ F (Ad5. β gal. Δ F has been deposited the ATCC under accession number VR2636) in
15 S8 cells containing alternative forms of TPL for enhancing the expression of fiber proteins, the protocol as described in Example 21 for preparing Ad5. β gal. Δ F in 211B cells was followed with the exception of pretreatment with 0.3 μ M dexamethasone for 24 hours as described above. Thus, viral particles with the wildtype Ad5 fiber protein on their surface
20 and containing the fiberless Ad5. β gal. Δ F genome were produced in 633 cells. Particles produced in 644 cells also contained the fiberless Ad5. β gal. Δ F genome, but had the chimeric 5T3H fiber protein, with the Ad3 fiber knob, on their surface. These viral preparations can be used to target delivery of the Ad5. β gal. Δ F, Ad5.GFP. Δ F, or other similarly
25 constructed fiberless genome with either wild-type or modified fibers.

-163-

EXAMPLE 23

Enhanced infectivity of dendritic cells by pseudotyped adenoviral particles

Bone marrow-derived dendritic cells were generated by culture of bone marrow cells from female Balb/C mice with GM-CSF and IL-4 (Inaba
5 *et al.* (1998) Isolation of dendritic cells *in* Current Protocols in Immunology, John Wiley & Sons, Inc. Philadelphia, 3.7.1-3.7.15). To confirm that the cultured cells expressed surface markers characteristic of dendritic cells, the cells were stained with fluorescently-conjugated antibodies directed against CD11c, CD80, and CD86 and analyzed by
10 fluorescence-activated cell sorting (FACS) analysis. Antibodies against the dendritic cell markers CD11c, CD80 and CD86 are commercially available, such as from eBioscience.

The primary dendritic cell cultures were infected with 100,000 viral particles/cell of Ad5.GFP. Δ F pseudotyped with either Ad5, Ad16, Ad19p,
15 Ad30, Ad35 or Ad37 fiber. The percent of cells positive for virus-induced GFP expression was determined by FACS analysis 48 hours after infection. All infections were performed in triplicate, and the mean \pm standard deviation was determined.

In agreement with previous experiments, Ad5.GFP. Δ F pseudotyped
20 with Ad5 fiber infected dendritic cells poorly with approximately 10% of cells positive for GFP expression, which is likely due to the lack of CAR expression on dendritic cells. In contrast, viruses carrying the Ad16, Ad19p, Ad30, Ad35 or Ad37 fiber proteins demonstrated enhanced infectivity of dendritic cells (approximately 49%, 46%, 37%, 26% and
25 50% of cells were GFP-positive), indicating that the fiber receptors for these serotypes are expressed on dendritic cells.

-164-

EXAMPLE 24

Subgroup D adenoviruses demonstrate selective infectivity

Sequence and phylogenetic analysis of adenovirus fiber DNA and amino acid sequence suggests that subgroup B and subgroup D viruses
5 bind different cellular receptors (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620). In addition, while subgroup B viruses (such as Ad16, Ad35 and Ad50), are capable of infecting a wide variety of cancer cell lines and primary cells, including endothelial cells, smooth muscle cells, synoviocytes, fibroblasts, amniocytes, dendritic cells, bone marrow
10 stroma cells, chondrocytes, myoblasts, melanocytes, follicle dermal papilla cells and hematopoietic stem cells (Havenga *et al.* (2002) *J. Virol.* 76:4612-4620), subgroup D viruses have a more selective tropism.

To determine whether select subgroup B (Ad3, Ad16 and Ad35) and subgroup D (Ad19p, Ad30 and Ad37) adenoviruses exhibit the same
15 cellular tropism, a panel of cancer cell lines were tested for their capacity to support Ad gene delivery. The cell lines used were PC-3 cells, HepG2 cells, LNCaP cells and DU 145 cells. These cell lines are available from the ATCC under accession numbers CRL-1435, HB-8065, CRL-10995 and HTB-81, respectively.

20 Each cell line was infected with either 1000, 5000 or 10,000 particles per cell of Ad5.GFP.ΔF pseudotyped with Ad5 (subgroup C), Ad3, Ad16, Ad19p, Ad30, Ad35 or Ad37 fiber. After 48 hours, virus-directed GFP expression was determined by FACS analysis. For PC-3 cells infected with 1000 particles per cell, little to no GFP expression was
25 detected in cells infected with viruses pseudotyped with subgroup D fibers Ad19p, Ad30 and Ad37. In contrast, GFP-expression was detected in approximately 40% of PC-3 cells infected with Ad16 and Ad35 fiber containing viruses. A similar pattern of GFP expression was found with cells infected at higher multiplicities of infection (MOIs). Approximately

-165-

80% of PC-3 cells infected with 5000 particles per cell of adenoviruses pseudotyped with Ad16 or Ad35 fiber were GFP positive, whereas only 2% of PC-3 cells were GFP positive when infected with Ad19p or Ad30 fiber pseudotyped viruses.

- 5 Similarly, in HepG2 cells, approximately 80% of cells were GFP-positive when infected with 5000 particles per cell of Ad16 or Ad35 fiber pseudotyped viruses, but less than 25% were GFP-positive when infected with either Ad19p or Ad30 fiber pseudotyped viruses. In addition, less than 10% of LNCaP cells were GFP-positive when infected with either
- 10 5000 or 10,000 particles per cell of Ad19p, Ad30 or Ad37 fiber containing adenoviruses, whereas Ad16 and Ad35 fiber directed GFP expression in approximately 65% of LNCaP cells. A similar pattern of infection was found in DU 145 cells. These results further demonstrate that subgroup B adenoviruses have a wider cellular tropism than subgroup
- 15 D viruses and provides additional evidence that subgroup B and subgroup D adenoviruses use different receptors for cell binding and infection.

EXAMPLE 25

20 **Immunization with adenovirus particles pseudotyped with Ad37 fiber results in T-cell stimulation**

- The following experiment was performed to determine whether immunization of mice with adenoviral particles pseudotyped with fiber protein from subgroup D adenovirus leads to stimulation of CD8+ T cells. Mice (eight in each experimental group, four in the vehicle (control)
- 25 group) were immunized by subcutaneous injection with 1×10^{10} particles of either Ad5.GFP.WT (Ad5 particles pseudotyped with Ad5 fiber) or Ad5.GFP.F37 (Ad5 particles pseudotyped with Ad37 fiber). Four weeks following inoculation, spleens were harvested to quantitate stimulation of T cells by determining the number of IFN- γ -positive CD8+ T cells.

-166-

To determine the percentage of activated CD8 + T cells in immunized mice, spleens were isolated and mechanically disrupted. Following lysis of red blood cells, 1×10^6 splenocytes were cultured for three hours in RPMI with 10% fetal calf serum and Golgiplug (BD Biosciences), in the presence or absence of 0.1 μ g/ml EGFP epitope peptide HYLSTQSAL or the irrelevant OVA peptide (SIINFEKL) as a control. Cells were then stained with an APC-conjugated anti-CD8 antibody (eBioscience), fixed and permeabilized using the Cytotfix/Cytoperm kit (BD Biosciences) and stained with a PE-conjugated antibody against IFN- γ . The cells were analyzed by fluorescence activated cell sorting (FACS) and the percentage of CD8 + cells positive for IFN- γ was determined ((number of CD8 + IFN- γ + cells divided by the total number of CD8 + T cells) \times 100).

Immunization with adenovirus particles pseudotyped with either Ad5 fiber or Ad37 fiber led to stimulation of CD8 + T cells, as indicated by production of IFN- γ in these cells. These results indicate adenovirus particles with Ad37 fiber are excellent vaccine candidates due to their ability to stimulate CD8 + T cells while avoiding transduction of liver cells.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

-167-

WHAT IS CLAIMED IS:

1. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber includes fiber from a subgroup D adenovirus for binding to dendritic cells, wherein the subgroup D adenovirus is selected from the group consisting of adenovirus serotype 8, 9, 10, 13, 15, 17, 19a, 19p, 20, 22-30, 32, 33, 36, 38, 39 and 42-49.

2. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to heparin sulfate proteoglycans (HSP) is reduced or eliminated compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber comprises fiber from Ad19p or Ad30, whereby HSP interaction is reduced.

3. An adenovirus particle, comprising a heterologous fiber or a portion thereof, whereby binding of the viral particle to dendritic cells is increased compared to a particle that expresses its native fiber, wherein:

the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber comprises fiber from a subgroup B adenovirus for binding the virus to dendritic cells, wherein the subgroup B adenovirus is selected from the group consisting of adenovirus serotype 7, 11, 14, 21, 34 or 50.

4. A particle of any of claims 1-3, wherein:

-168-

the fiber is chimeric and comprises an N-terminal portion from a fiber of a subgroup C adenovirus; and

the N-terminal portion is sufficient to increase incorporation into the particle compared to in its absence.

5. The particle of claim 1 or 2, wherein the fiber is a chimeric fiber that includes a sufficient portion of a subgroup D adenovirus fiber to target dendritic cells.

6. The particle of any of claims 1-5, wherein the subgroup C virus is selected from the group consisting of adenovirus serotype 1, 2, 5, and 6.

7. The particle of any of claims 1-6, wherein the fiber is further modified to reduce any interaction with CAR.

8. The particle of any of claims 1 and 3-7, wherein the fiber is modified to reduce any interaction with heparin sulfate proteoglycans (HSP).

9. The particle of any of claims 1-8, wherein the capsid includes further modifications that alter interaction with α_v integrin.

10. The particle of any of claims 1 or 4-9, wherein the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber is from Ad19p.

11. The particle of claim 2 or claim 10, wherein the Ad19p fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 34 to target the particle to dendritic cells.

12. The particle of claim 11, wherein the Ad19p fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 34 to target the particle to dendritic cells, but exhibits reduced binding to HSP compared to a subgroup C fiber.

-169-

13. The particle of any of claims 10-12, wherein the fiber is chimeric and includes a portion of a subgroup C adenovirus.

14. The particle of any of claims 1 or 4-9, wherein the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the fiber is from Ad30.

15. The particle of claim 14, wherein the Ad30 fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 36 to target the particle to dendritic cells.

16. The particle of claim 14, wherein the Ad30 fiber comprises at least a sufficient number of amino acids set forth as SEQ ID NO. 36 to target the particle to dendritic cells, but exhibits reduced binding to HSP compared to a subgroup C fiber.

17. The particle of any of claims 14-16, wherein the fiber is chimeric and includes a portion of a subgroup C adenovirus.

18. An adenovirus particle of any of claims 8-17, comprising a mutation in a CAR-binding region of the capsid to decrease CAR binding.

19. An adenovirus particle of any of claims 1-18, comprising a mutation in the α_v integrin-binding region of the capsid, whereby binding to the integrin is eliminated or reduced.

20. The adenovirus particle of claim 1, 2, 4-13, 18 or 19, wherein the Ad19p fiber is modified by replacing the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber.

21. The adenovirus particle of claim 14-17, wherein the Ad30 fiber is modified by replacing the N-terminal 15, 16 or 17 amino acids with the 15, 16 or 17 amino acids of an Ad2 or Ad5 fiber.

22. The adenovirus particle of claim 7 or claim 18, wherein the CAR-binding region of the capsid that is modified is on a fiber knob.

-170-

23. The adenovirus particle of claim 22, wherein the fiber protein further comprises one or more further modifications that reduce or eliminate interaction of the resulting fiber with HSP.

24. The adenovirus particle of claim 23, wherein the capsid further comprises a ligand, whereby the particle binds to a receptor for the ligand.

25. The adenovirus particle of claim 24, wherein the ligand is included in the knob region of the fiber.

26. The adenovirus particle of claim 24, wherein the ligand is inserted into the fiber or it replaces a portion of the fiber.

27. A particle of any of claims 1-26, further comprising a heterologous nucleic acid in the genome thereof, wherein the heterologous nucleic acid encodes an antigen or a product that alters dendritic cell activity.

28. The particle of claim 27, wherein the antigen is a tumor antigen or an antigen from a pathogen.

29. A composition formulated for administration to a subject comprising a particle of any of claims 1-28.

30. A composition of claim 29 formulated for intramuscular or IV or parenteral administration.

31. A composition of claim 29 or claim 30 that is a vaccine.

32. An immunotherapeutic method, comprising administering a composition of any of claims 29-31 to a subject.

33. A method of delivering viral particles to dendritic cells, comprising:

contacting a composition with cells that comprise dendritic cells, whereby viral particles bind to dendritic cells, wherein the composition contains a viral particle of any of claims 1 and 3-28 or an adenovirus particle that comprises a fiber from Ad37 for targeting the

-171-

particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

infusing the composition into a subject.

34. The method of claim 33, wherein the cells are removed from the subject prior to contacting.

35. The method of claim 33, wherein the cells comprise immune cells.

36. The method of claim 33, wherein the cells are bone marrow cells.

37. A nucleic acid molecule encoding a viral particle of any of claims 1-28.

38. The nucleic acid molecule of claim 37 that comprises an adenovirus vector.

39. The nucleic acid molecule of claim 37 or claim 38 that comprises heterologous nucleic acid.

40. A cell, comprising the nucleic acid of any of claims 37-39.

41. The cell of claim 40 that is a dendritic cell.

42. A method of treatment, comprising administering a cell to a subject who has an immune cell disorder, cancer or an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

43. The method of claim 32 or 42, wherein the subject is infected with a pathogen, has a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.

44. A method of targeting an adenovirus particle to dendritic cells, comprising replacing all or a portion of the native fiber of the adenovirus with an adenovirus subgroup D fiber.

-172-

45. The method of claim 44, wherein:
the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the subgroup D adenovirus is selected from the group consisting of adenovirus serotype 8, 9, 10, 13, 15, 17, 19a, 19p, 20, 22-30, 32, 33, 36, 37, 38, 39 and 42-49.

46. A method of targeting an adenovirus particle to dendritic cells, comprising replacing all or a portion of the native fiber of the adenovirus with an adenovirus subgroup B fiber.

47. The method of claim 46, wherein:
the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus; and

the subgroup B adenovirus is selected from the group consisting of adenovirus serotype 3, 7, 11, 14, 16, 21, 34, 35, or 50.

48. The method of claim 45 or claim 47, wherein the subgroup C virus is selected from the group consisting of adenovirus serotypes 1, 2, 5, and 6.

49. The method of claim 45 or claim 47, wherein the fiber is further modified to reduce any interaction with CAR.

50. The method of claim 49, wherein the fiber is further modified to reduce any interaction with heparin sulfate proteoglycans (HSP).

51. The method of claim 50, wherein the capsid includes further modifications that alter interaction with α_v integrin.

52. Use of an adenovirus particle for treatment of a disorder or disease, wherein the particle is a particle of any of claims 1-28 or is an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

-173-

53. The use of claim 52, wherein the disease or disorder is an immune cell disorder, cancer or an infection.

54. Use of an adenovirus particle for preparation of a medicament for the treatment of an immune cell disorder, cancer or an infection, wherein the particle is a particle of any of claims 1-28 or is an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

55. Use of a cell for treatment of disease or disorder selected from an immune cell disorder, cancer and an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

56. The use of claim 55, wherein the disorder is a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.

57. Use of a cell for the preparation of a medicament for the treatment of a disease or disorder selected from among an immune cell disorder, cancer and an infection, wherein the cell is a cell of claim 41 or a dendritic cell containing an adenovirus particle that comprises a fiber from Ad37 for targeting the particle to dendritic cells and the adenovirus (Ad) particle, except for the fiber, is from a subgroup C adenovirus.

58. The use of claim 57, wherein the disease or disorder is a tumor, an inflammatory disorder, allergies, asthma or an autoimmune disease.

1 / 35

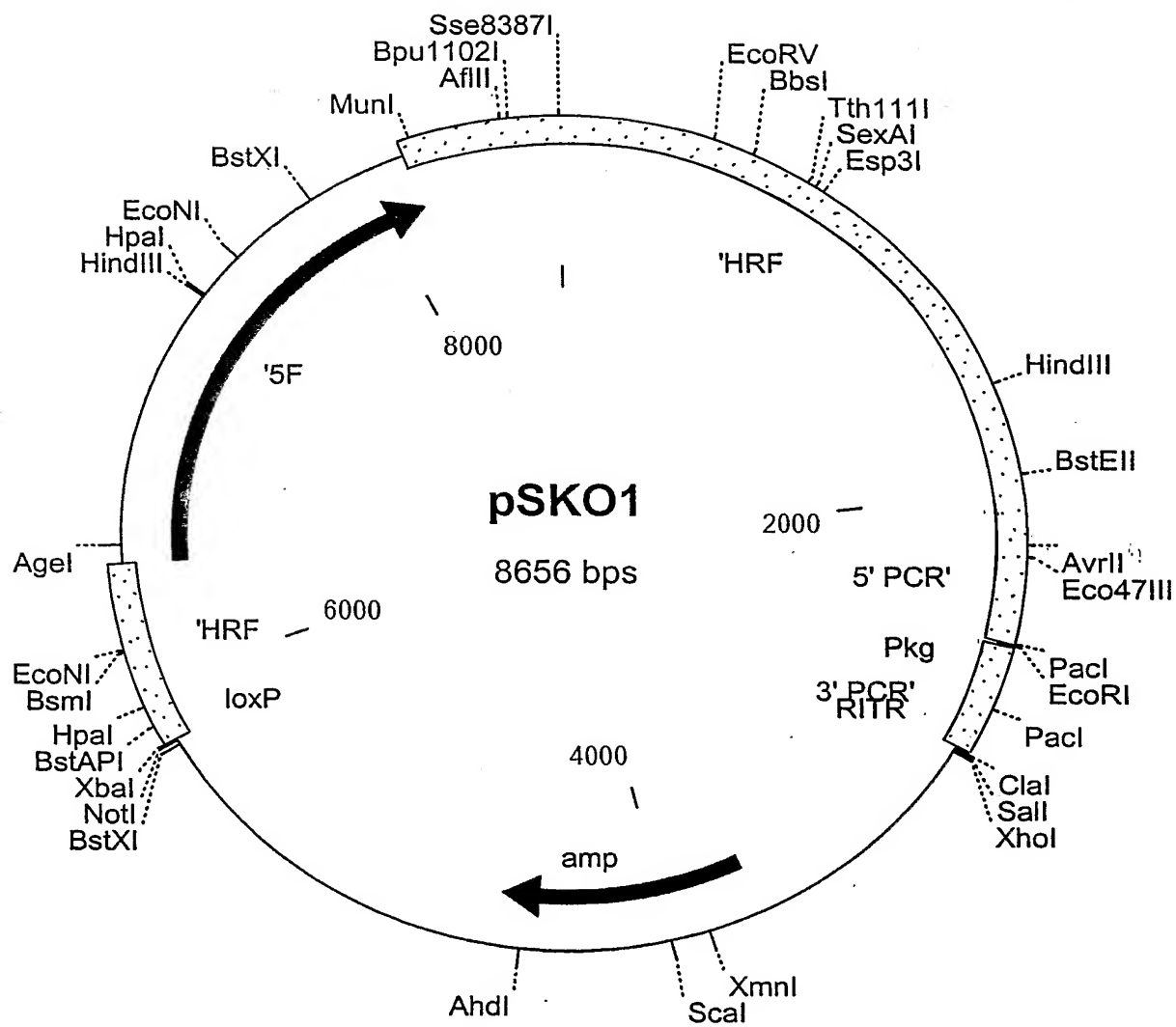
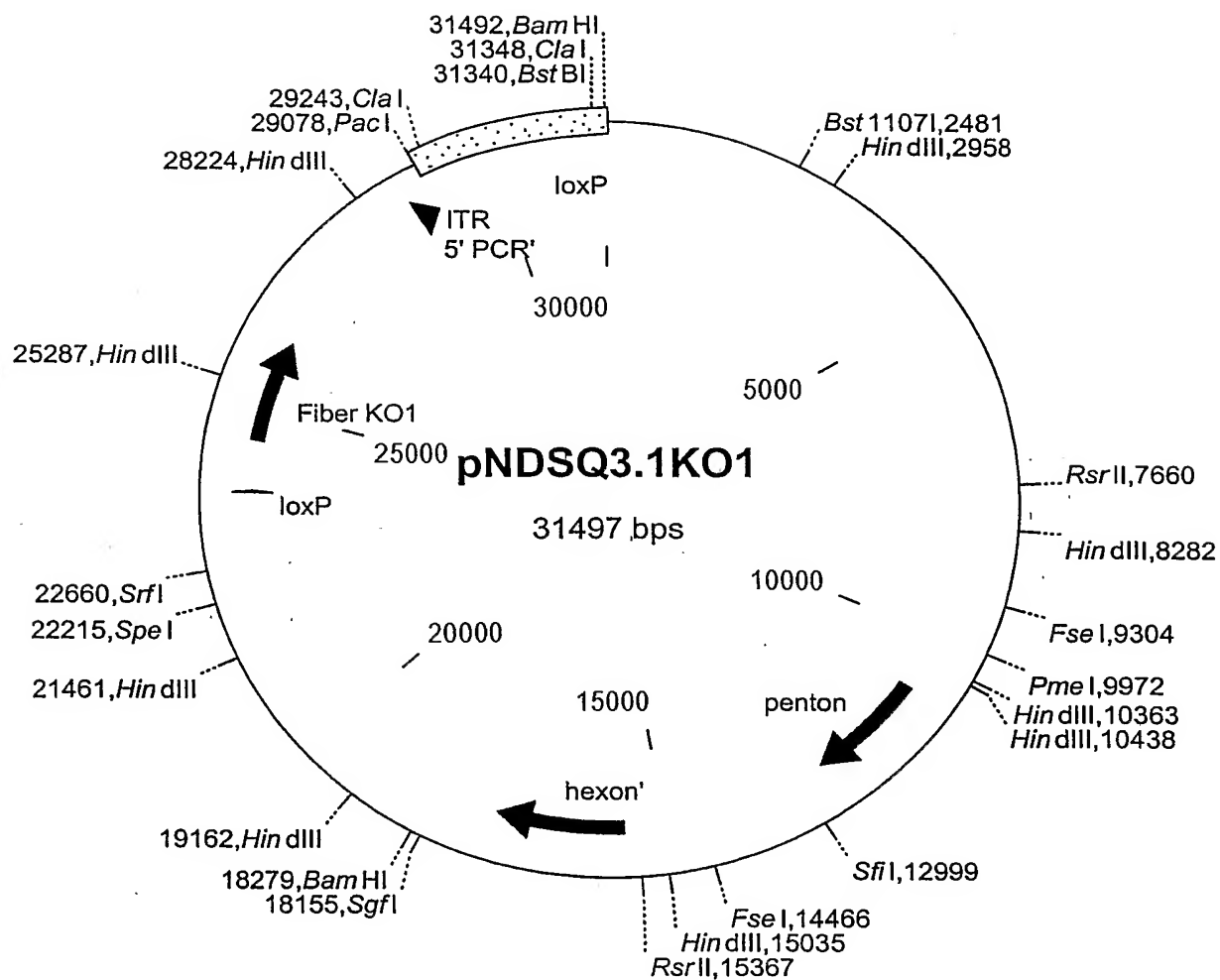


FIG. 1

2 / 35

**FIG. 2**

3 / 35

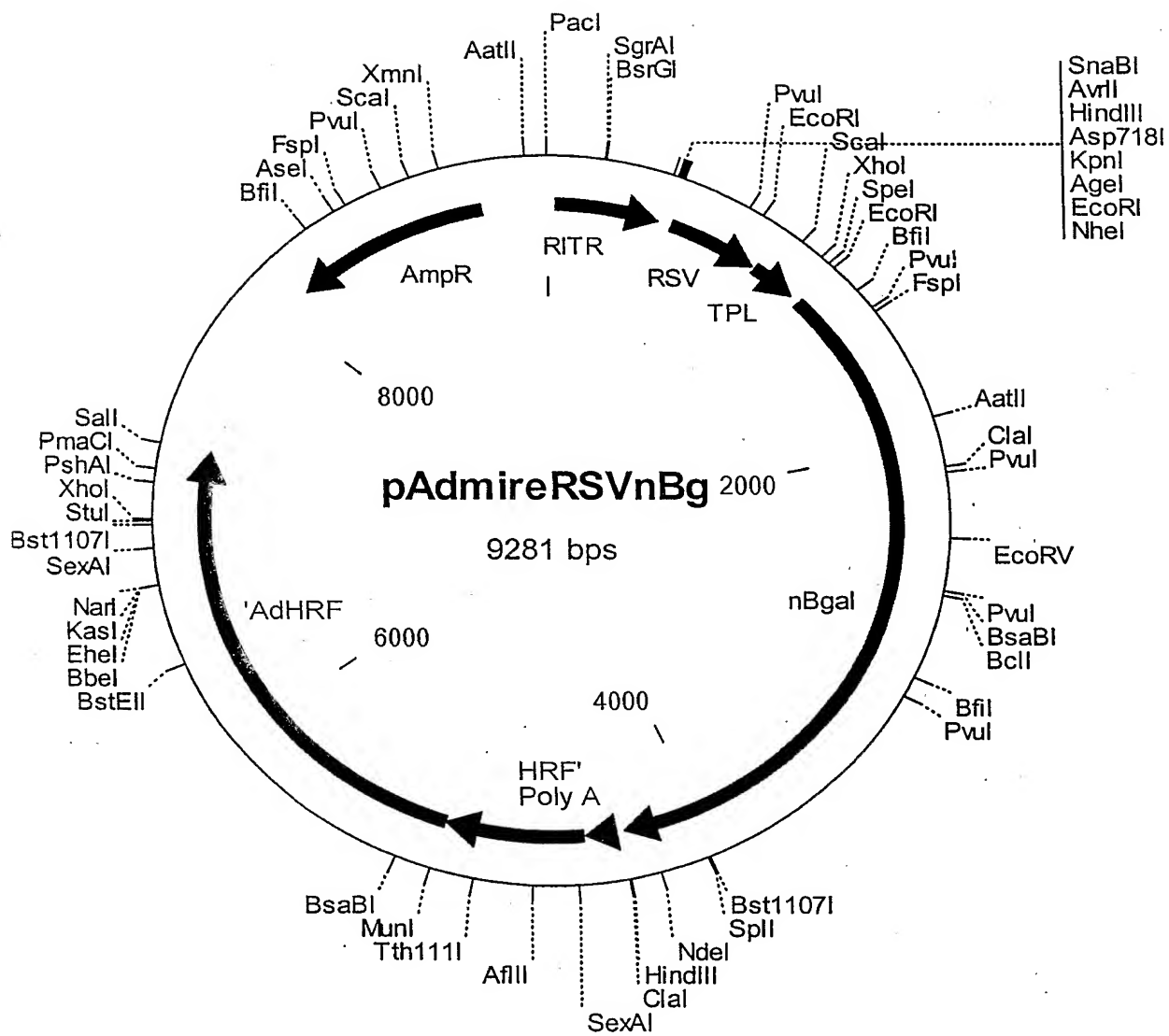
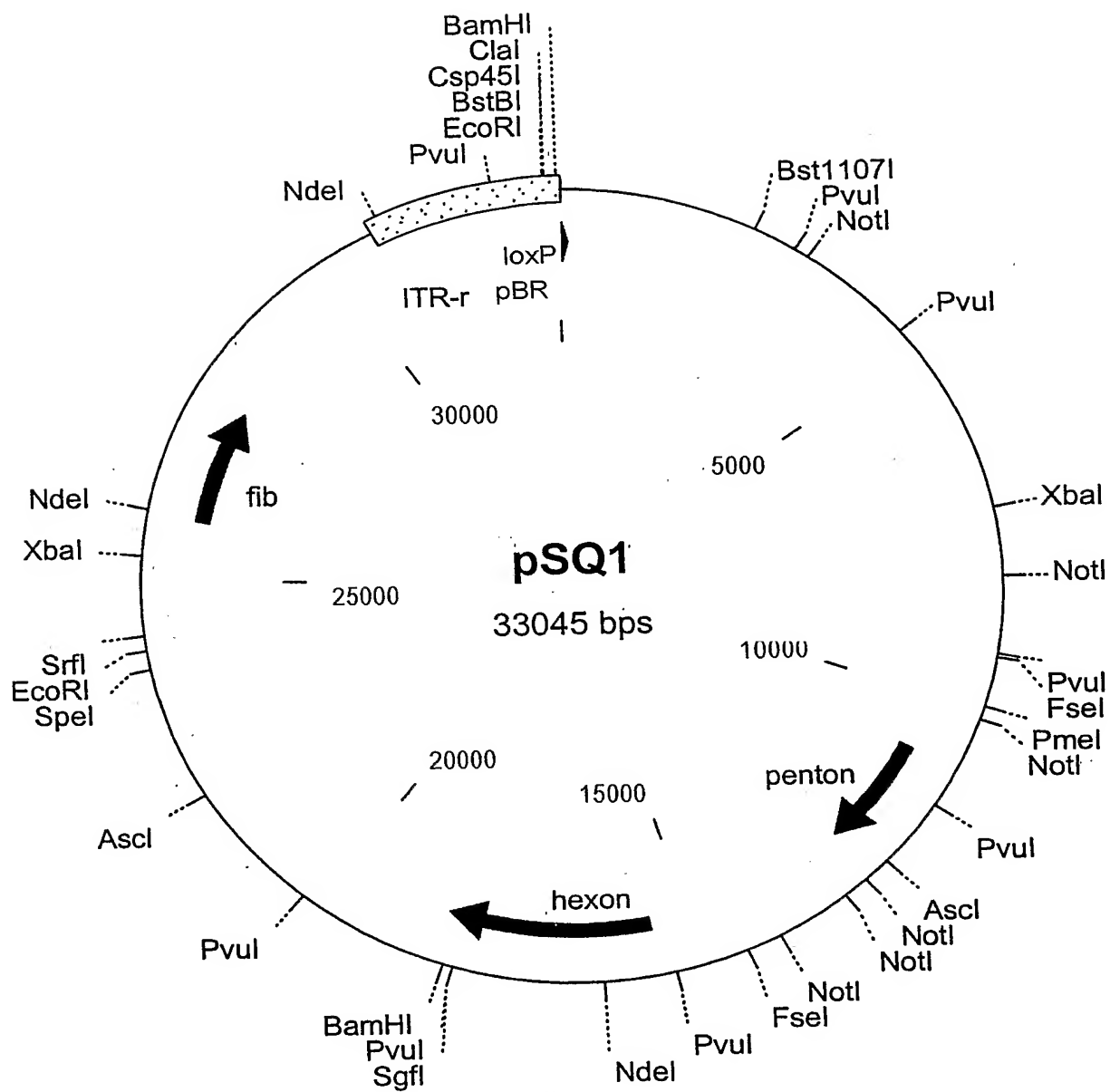


FIG. 3A

4 / 35

**FIG. 3B**

5/35

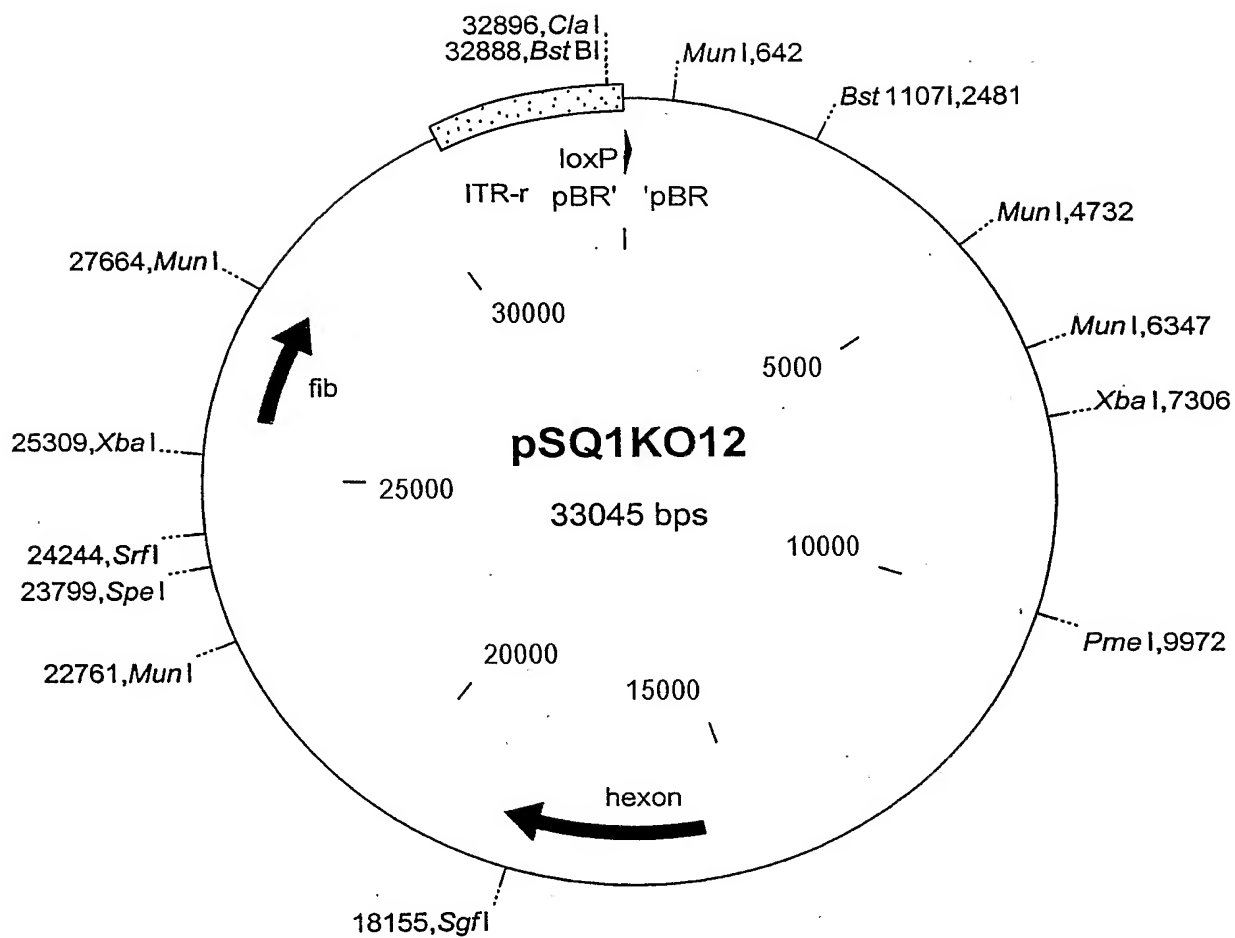


FIG. 3C

6/35

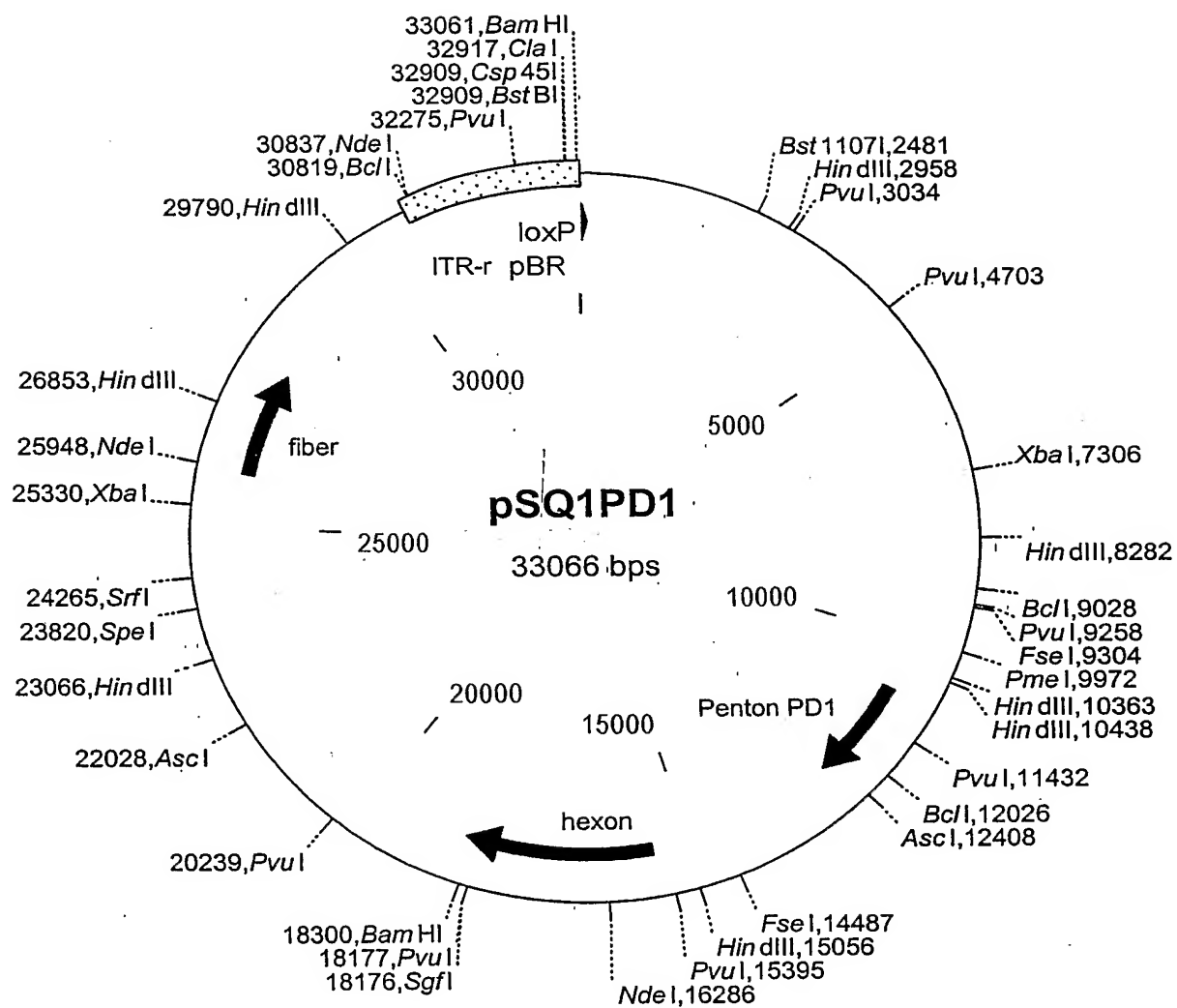


FIG. 4

7/35

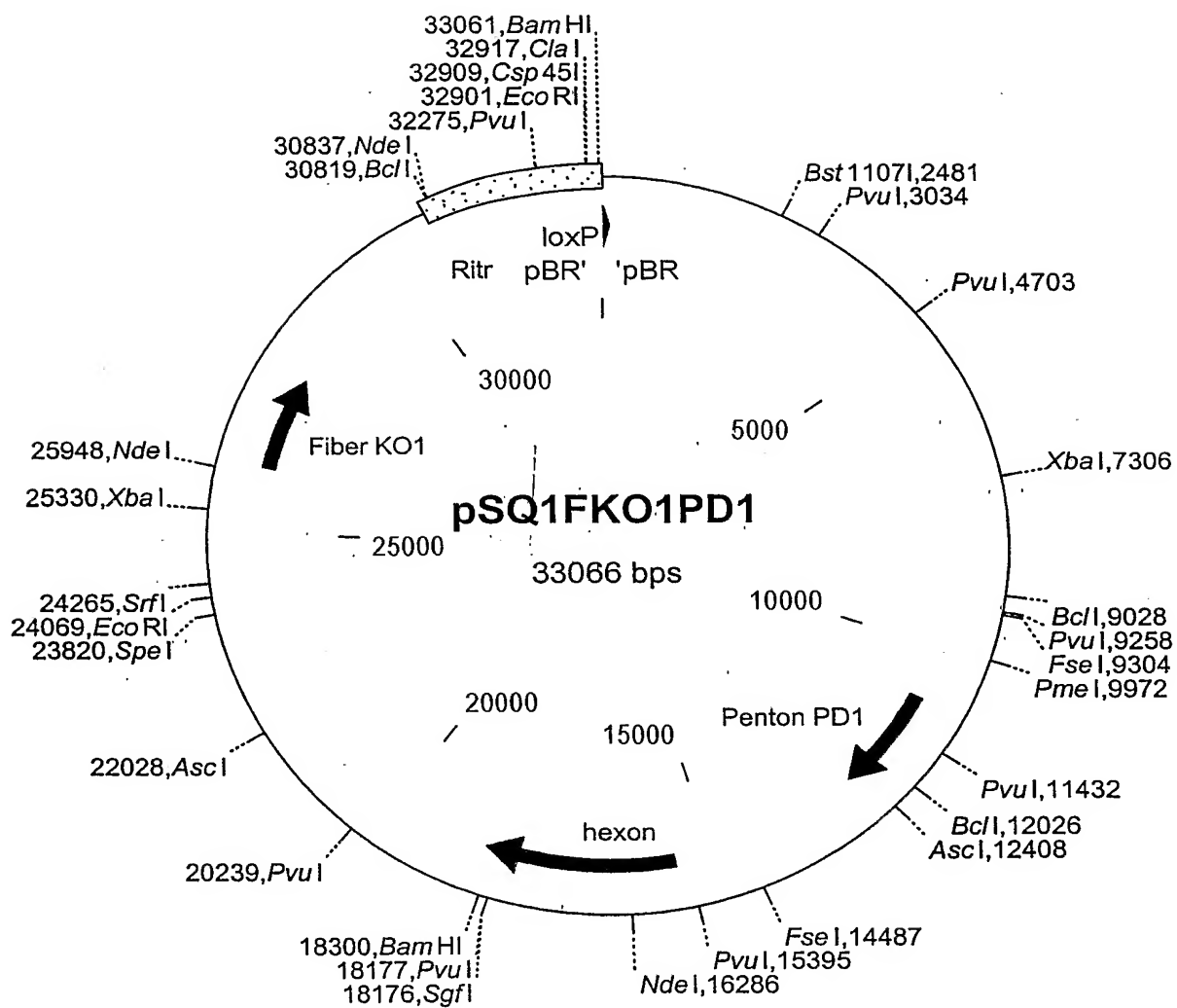


FIG. 5A

8/35

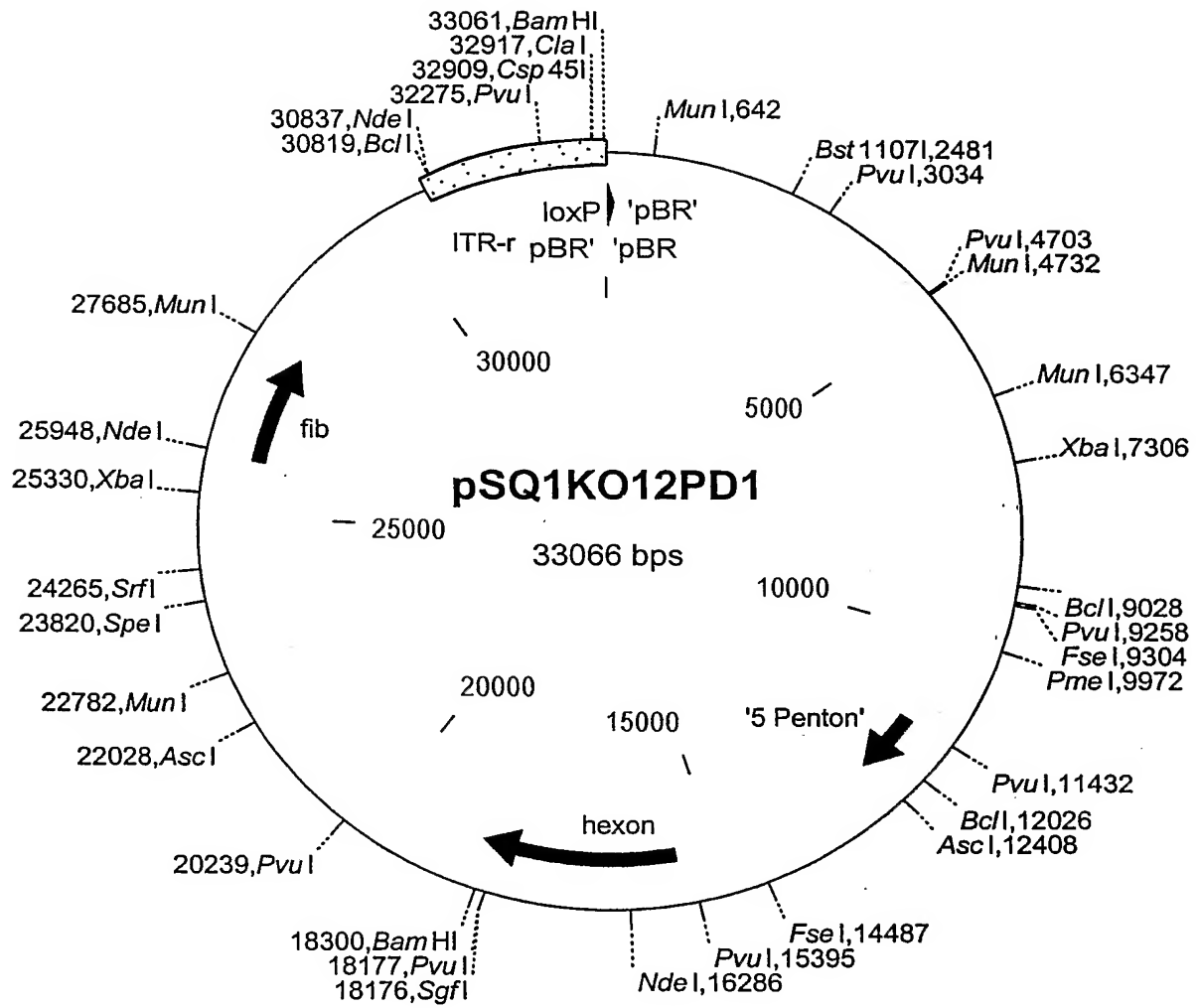
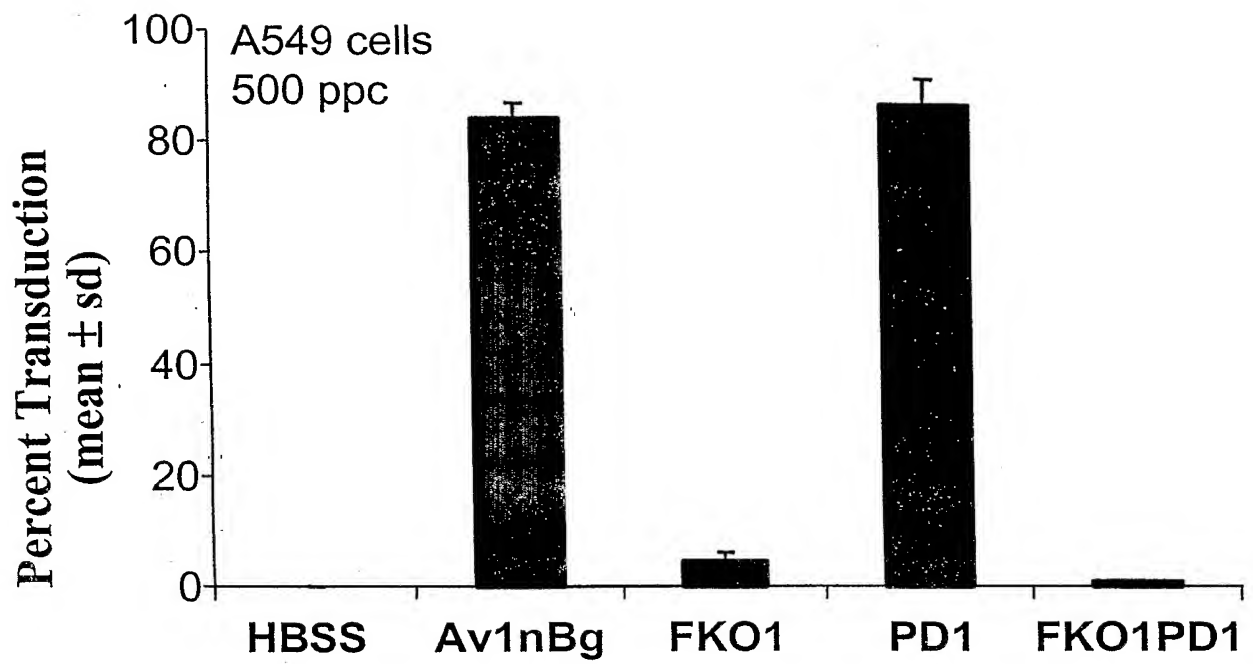
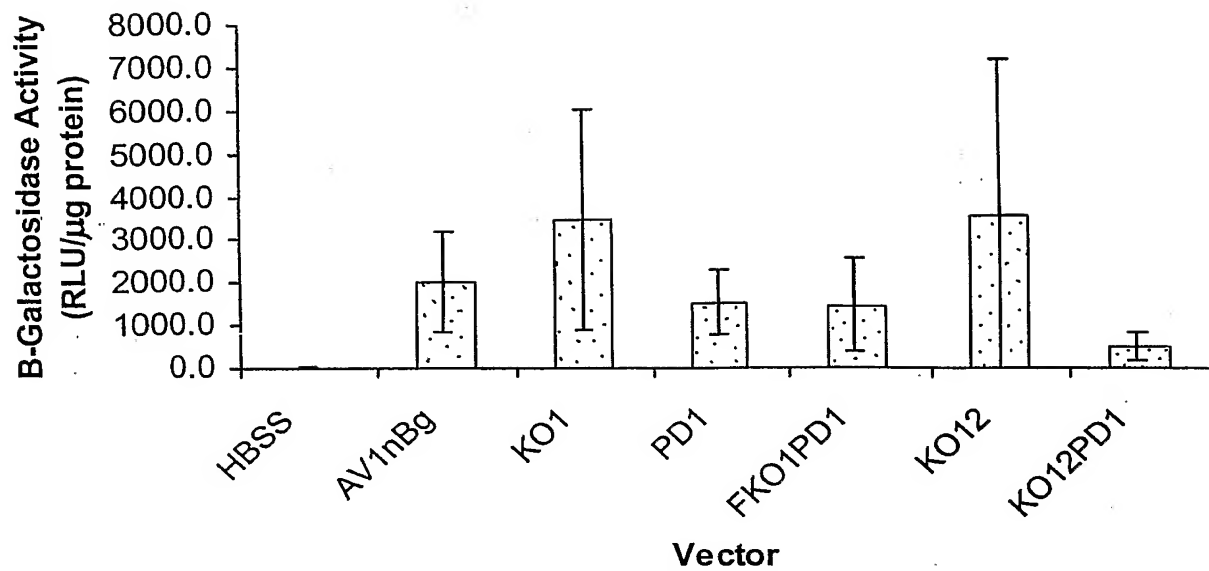
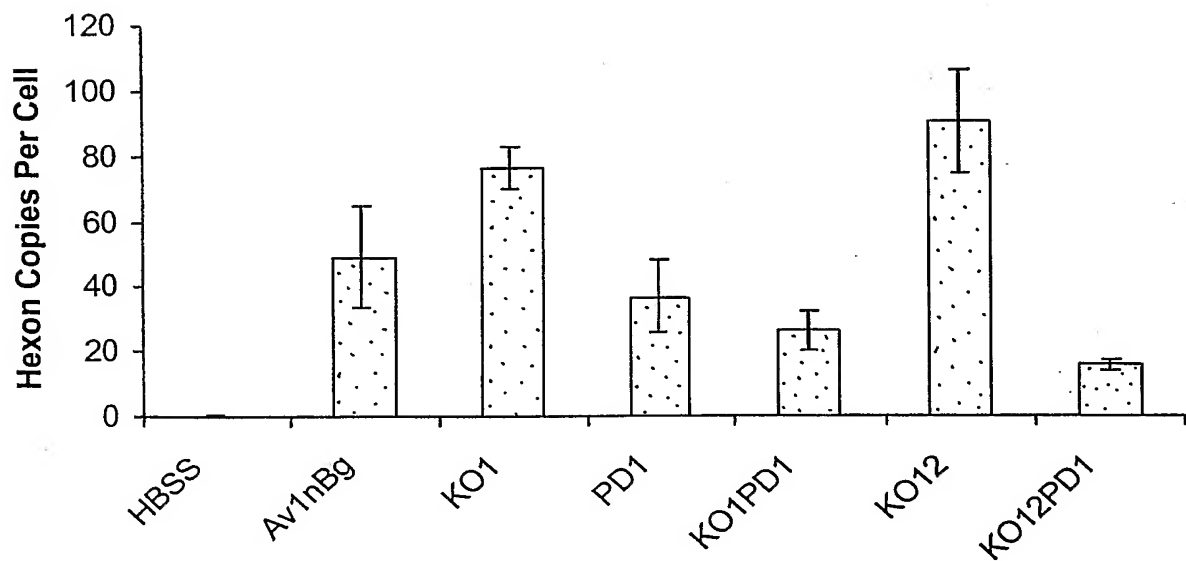


FIG. 5B

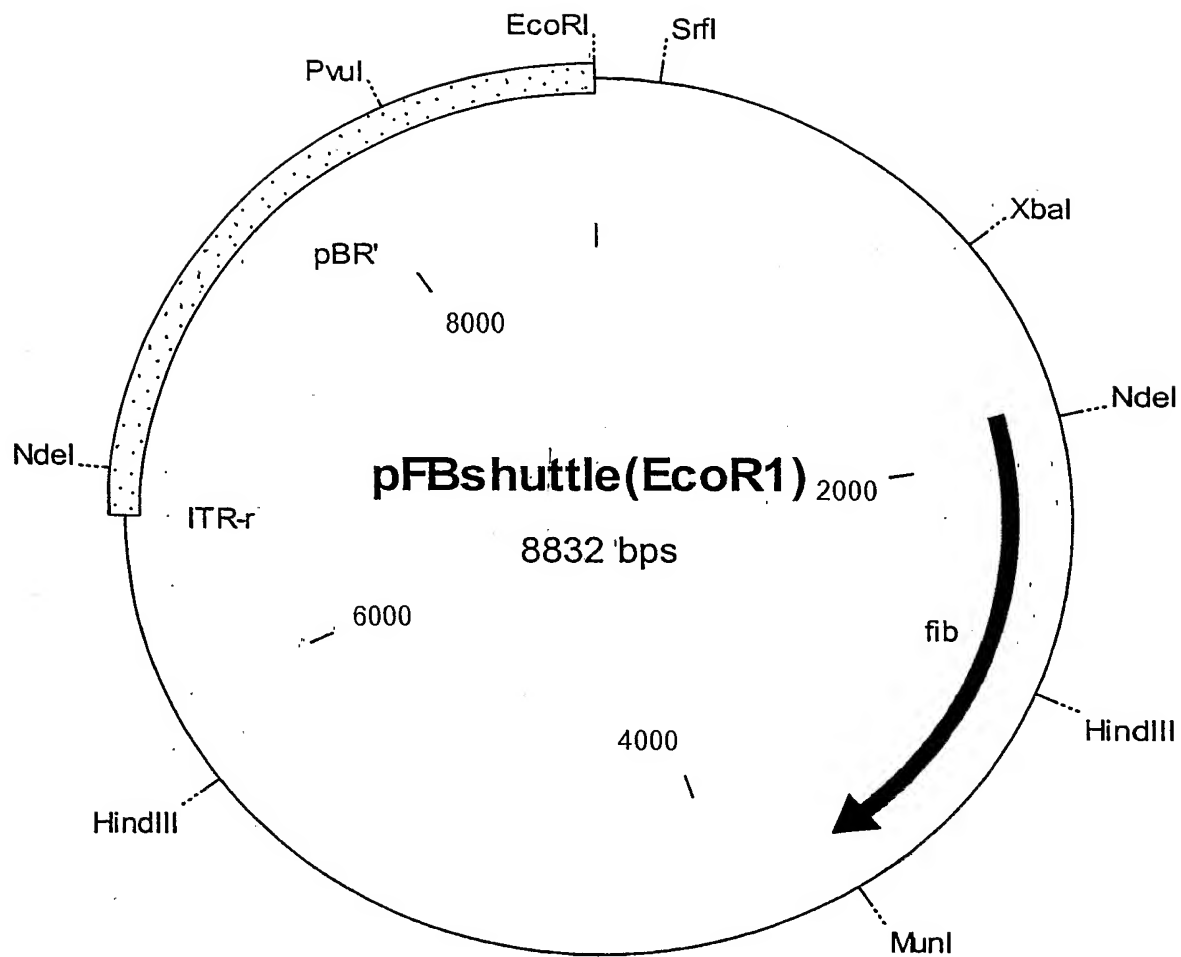
9/35

**FIG. 6**

10/35

**FIG. 7A****FIG. 7B**

11/35

**FIG. 8**

12 / 35

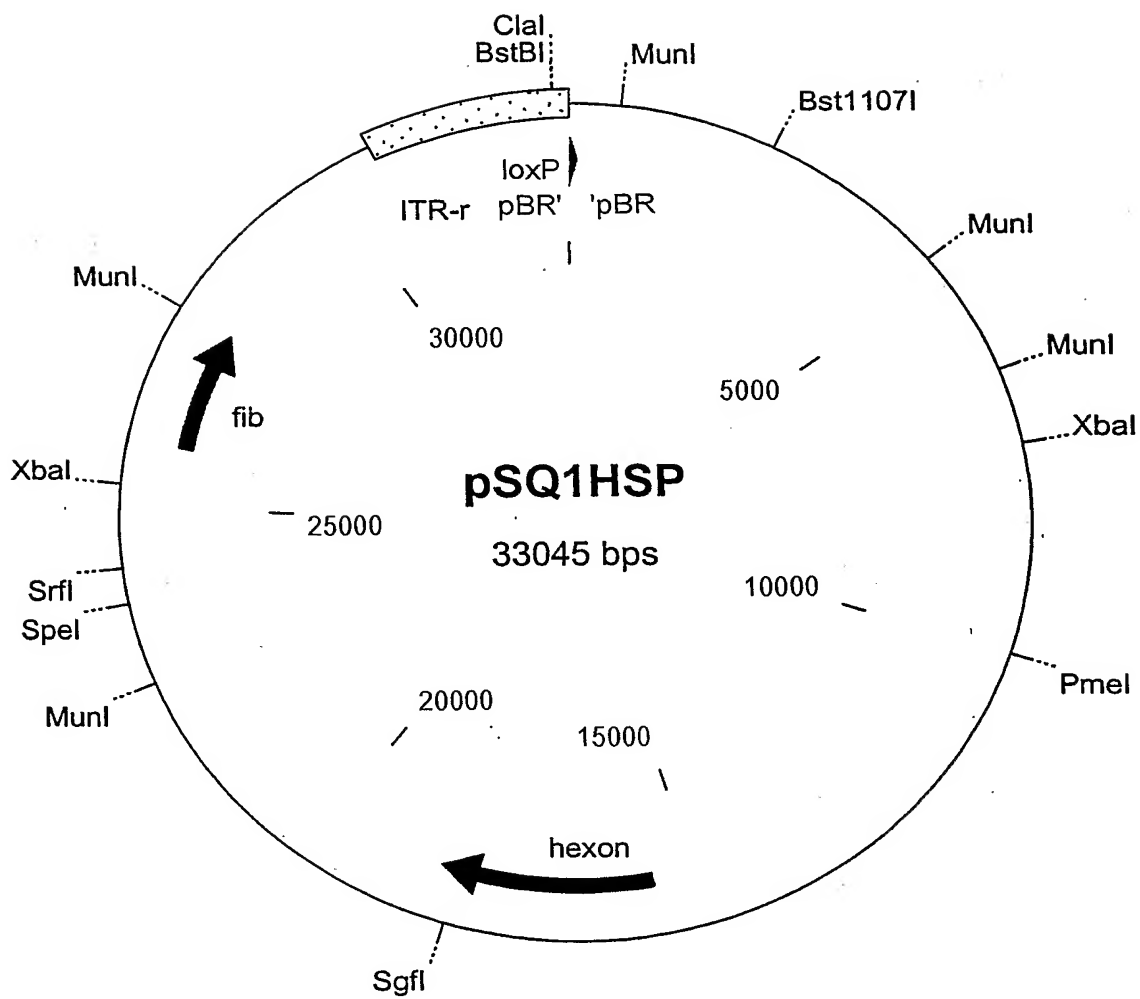
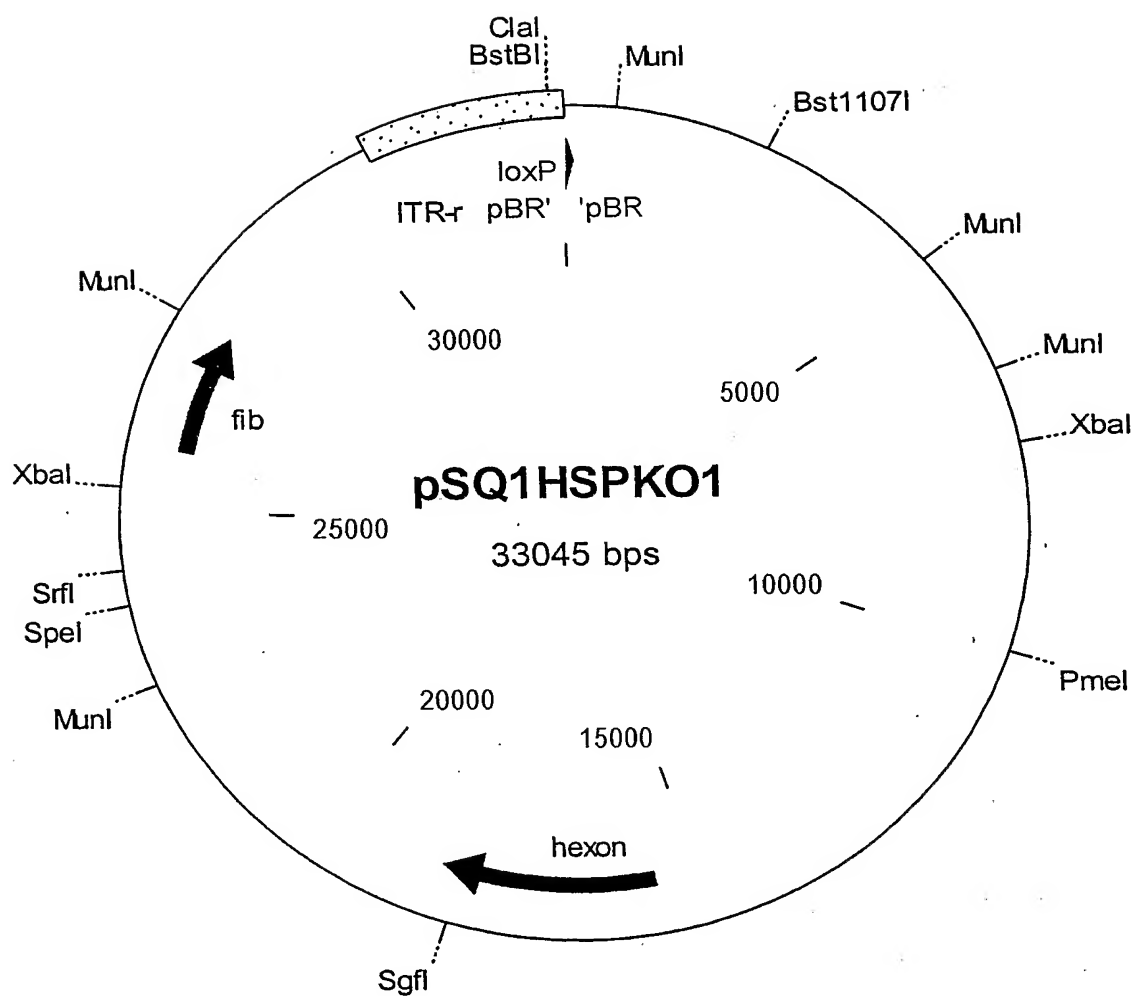


FIG. 9

13/35

**FIG. 10**

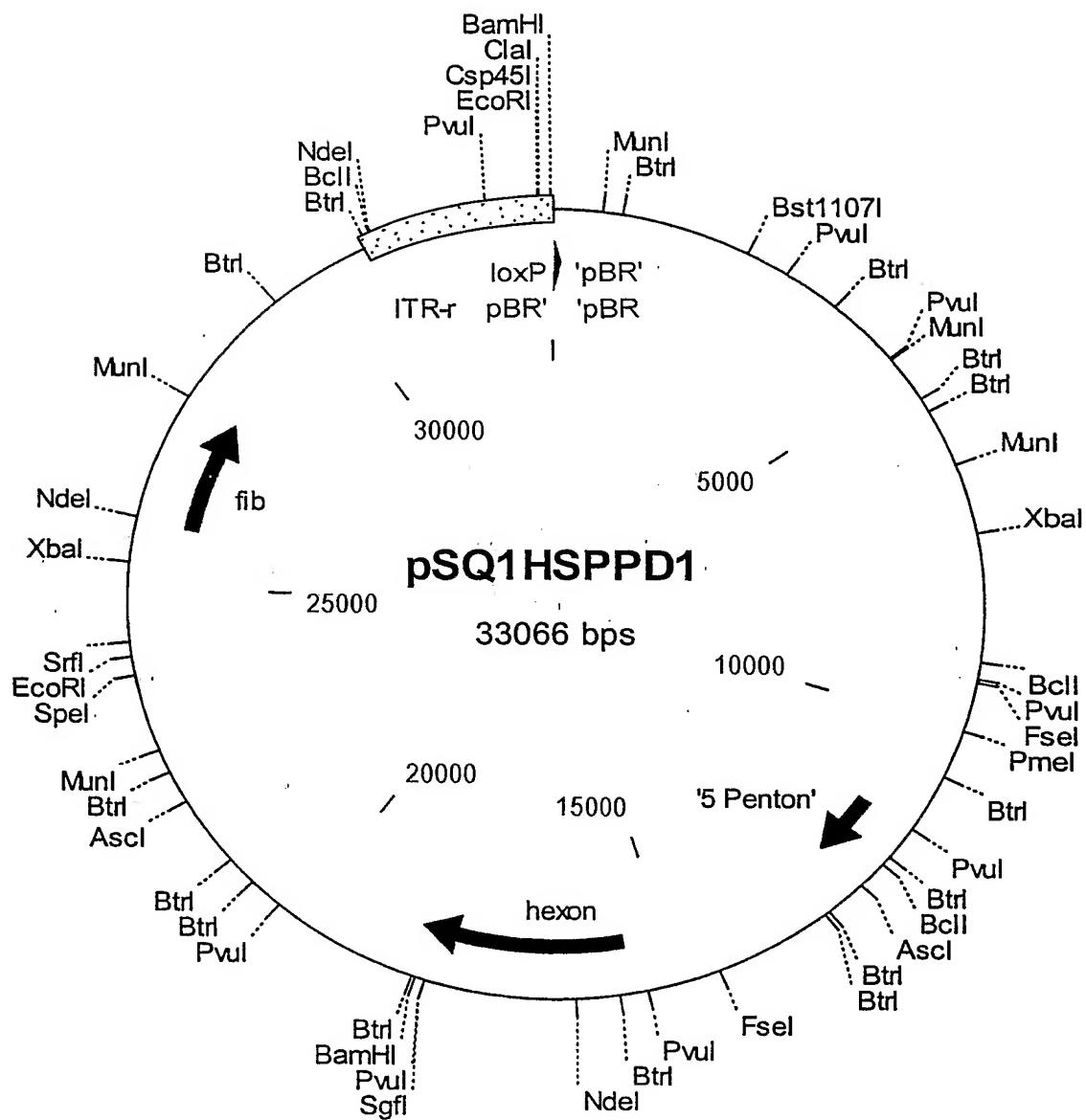


FIG. 11

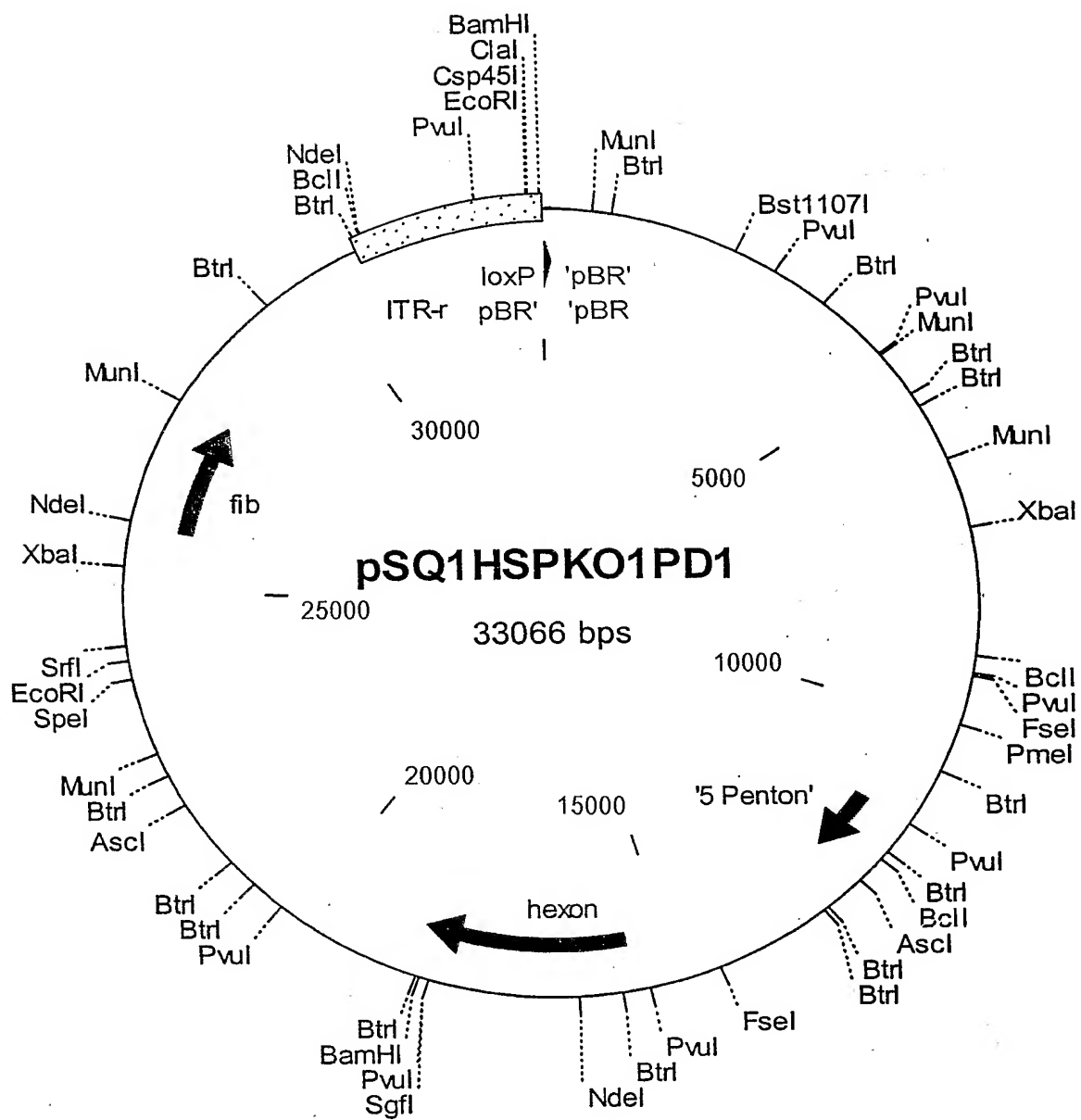
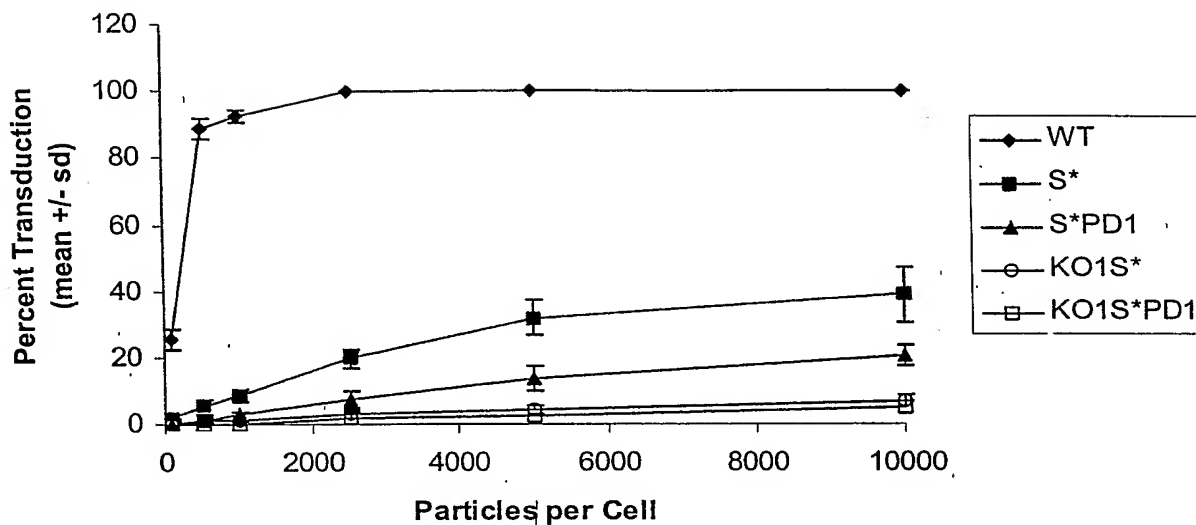
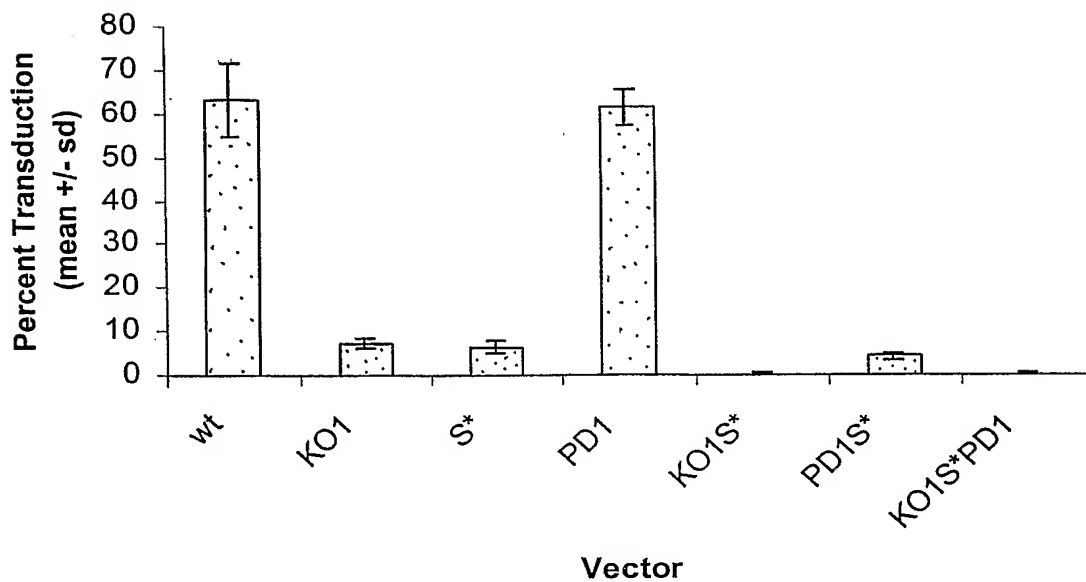


FIG. 12

16/35

**FIG. 13A****FIG. 13B**

17/35

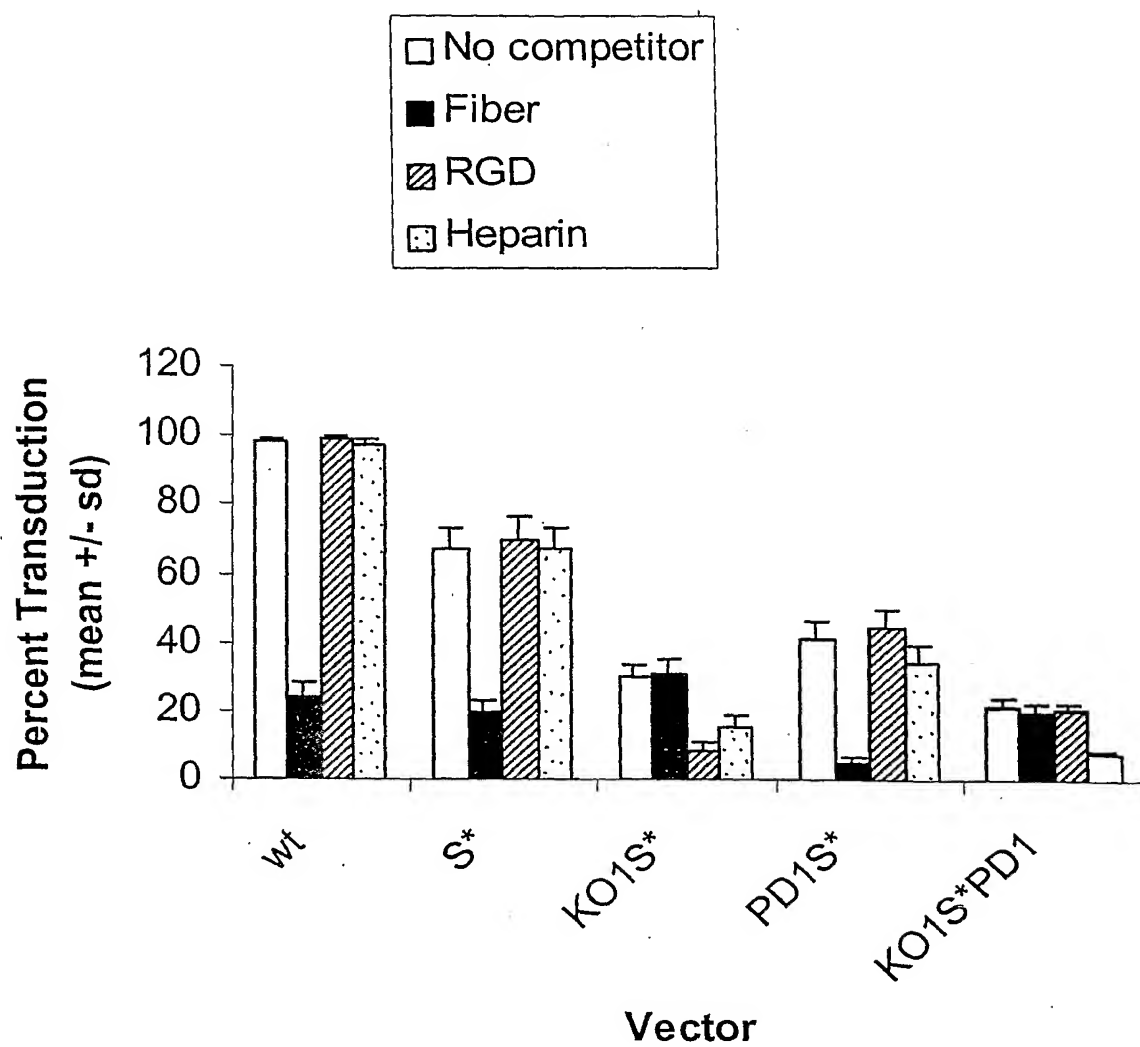
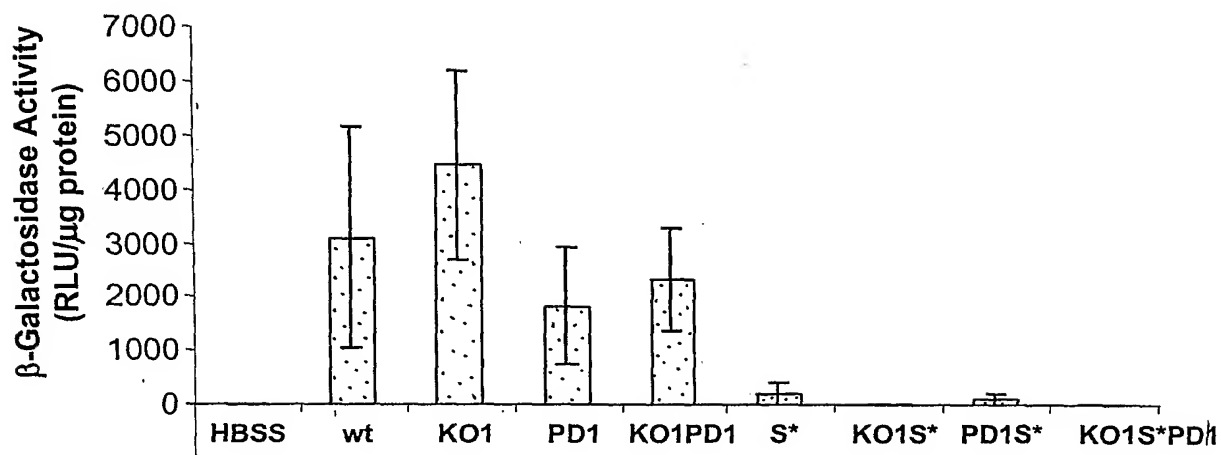
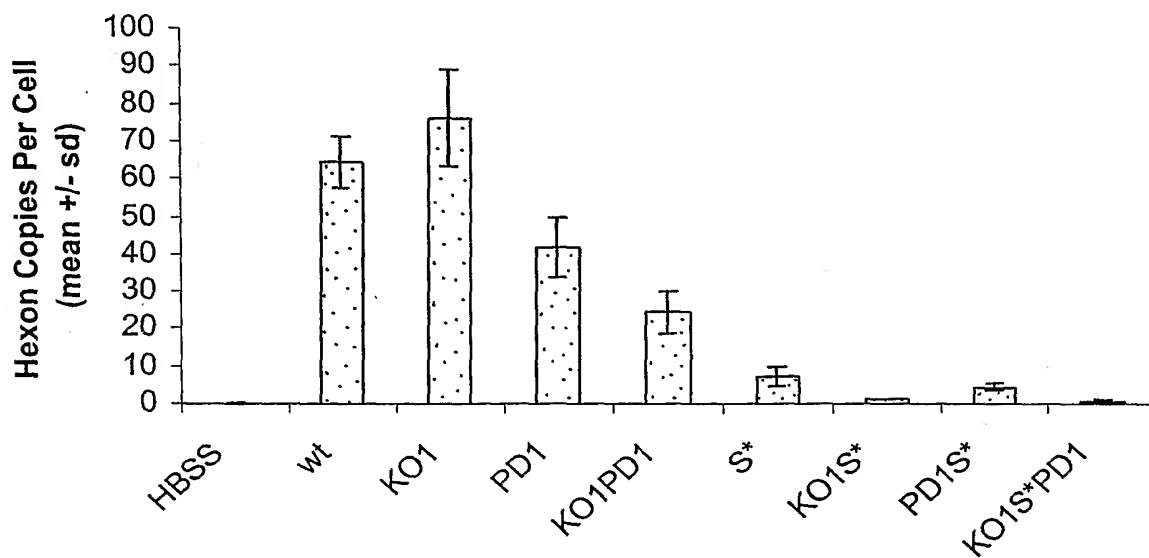


FIG. 13C

18/35

**FIG. 14A****FIG. 14B**

19/35

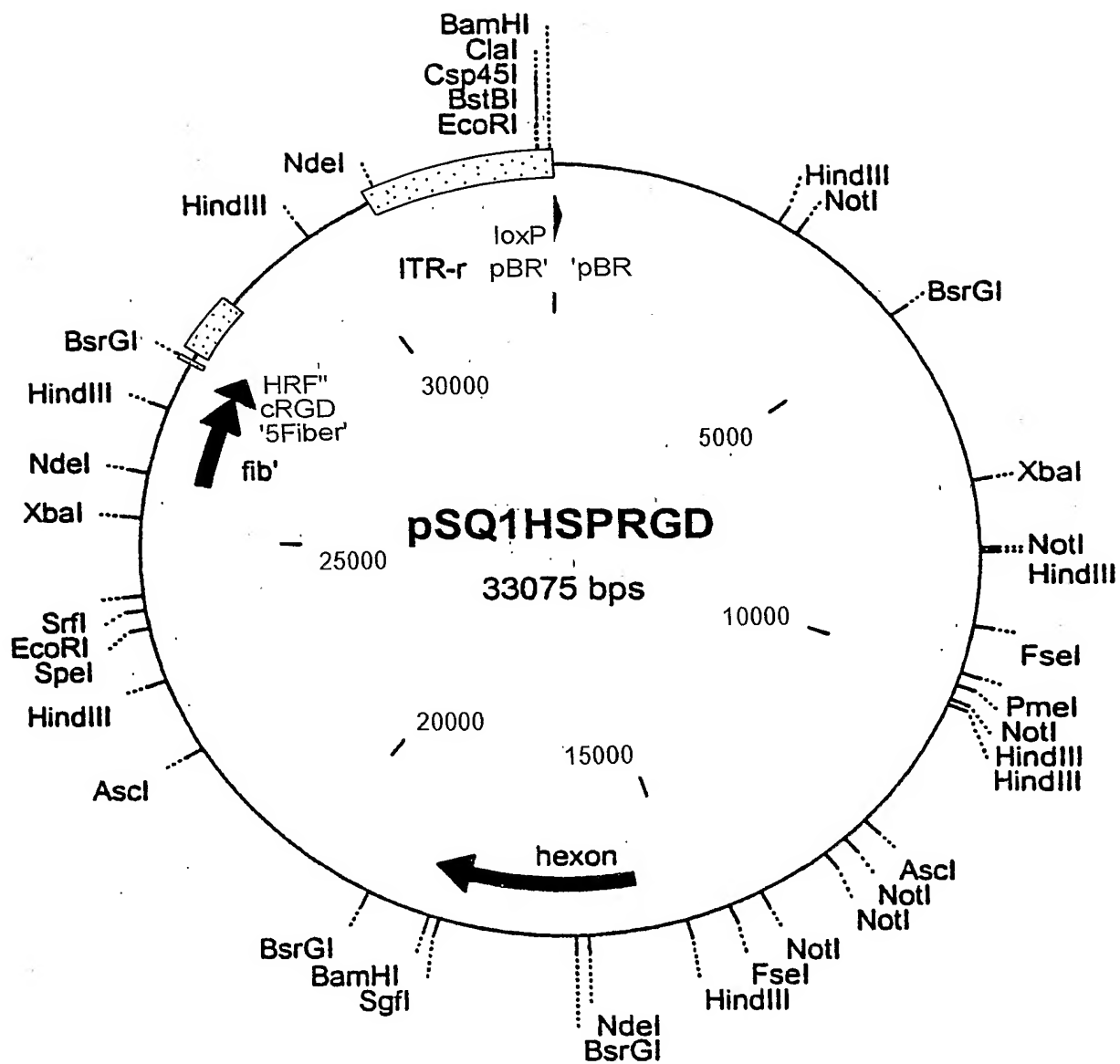


FIG. 15A

20/35

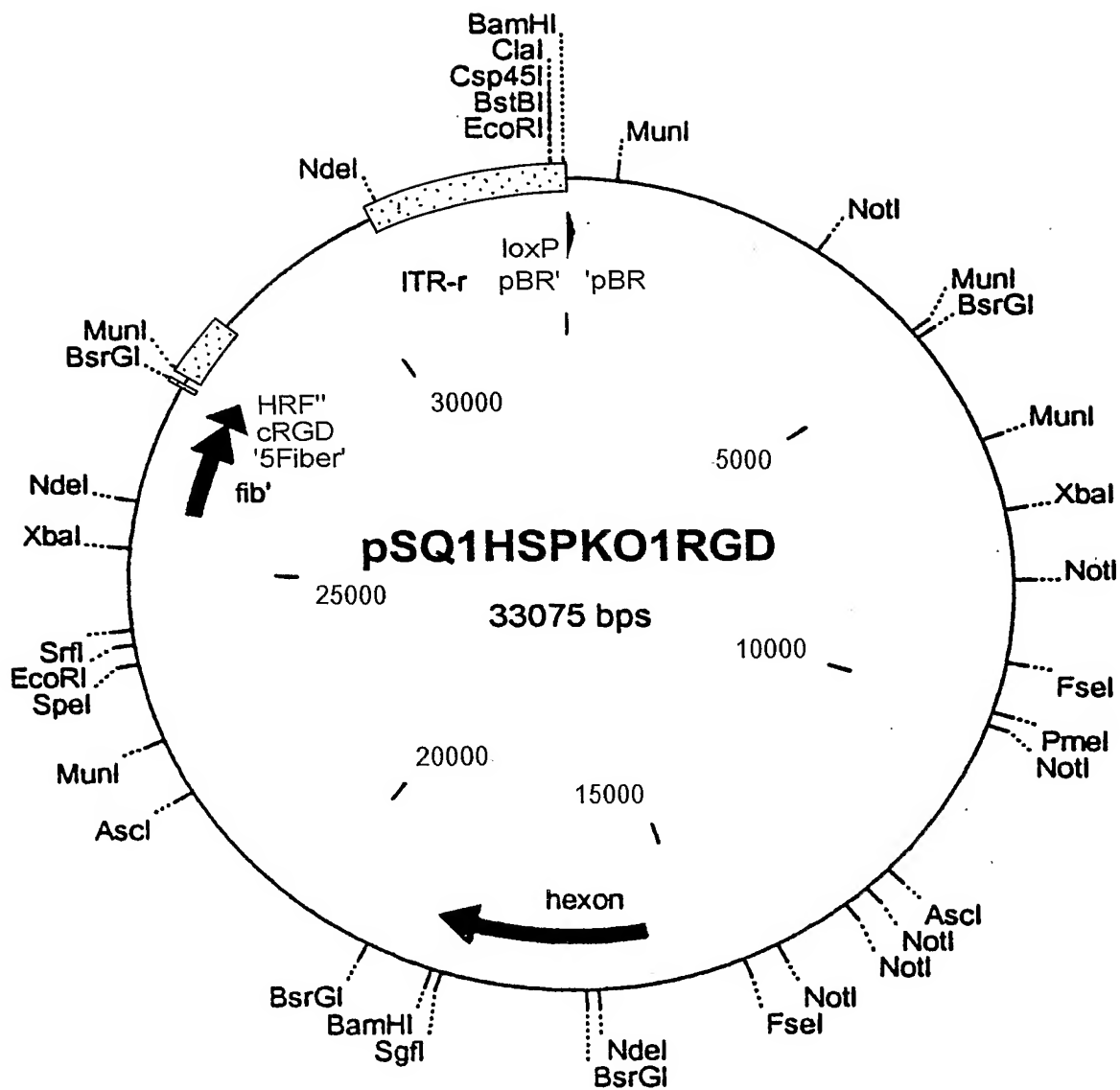


FIG. 15B

21/35

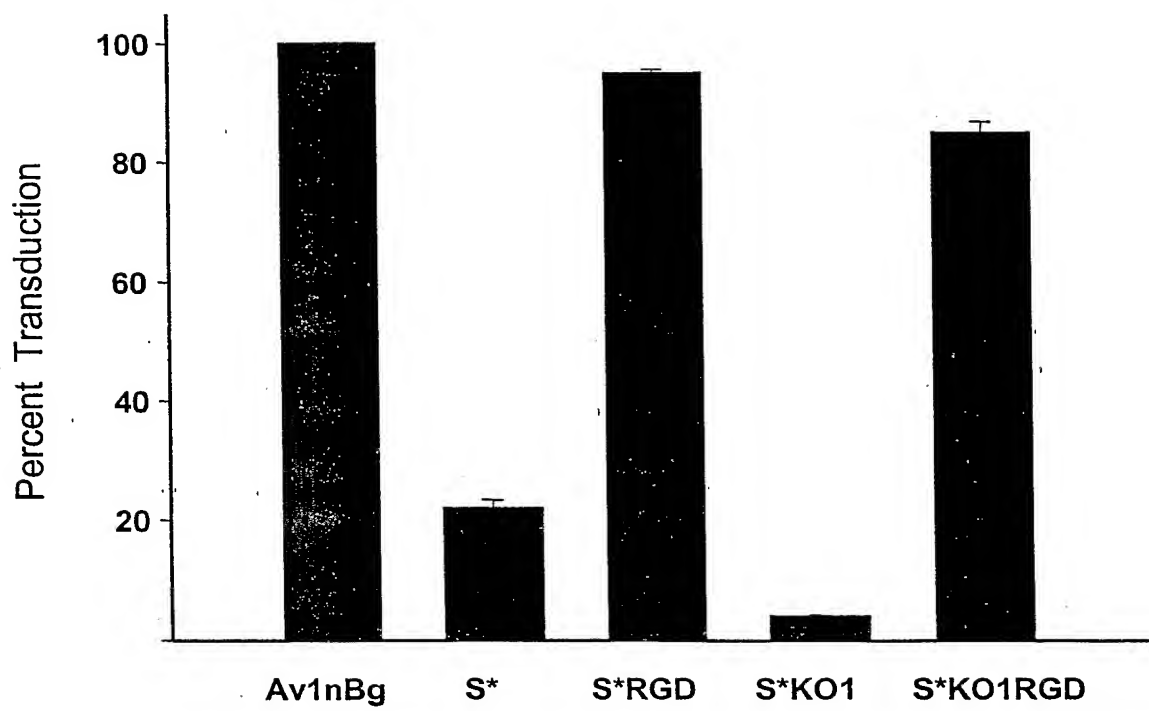
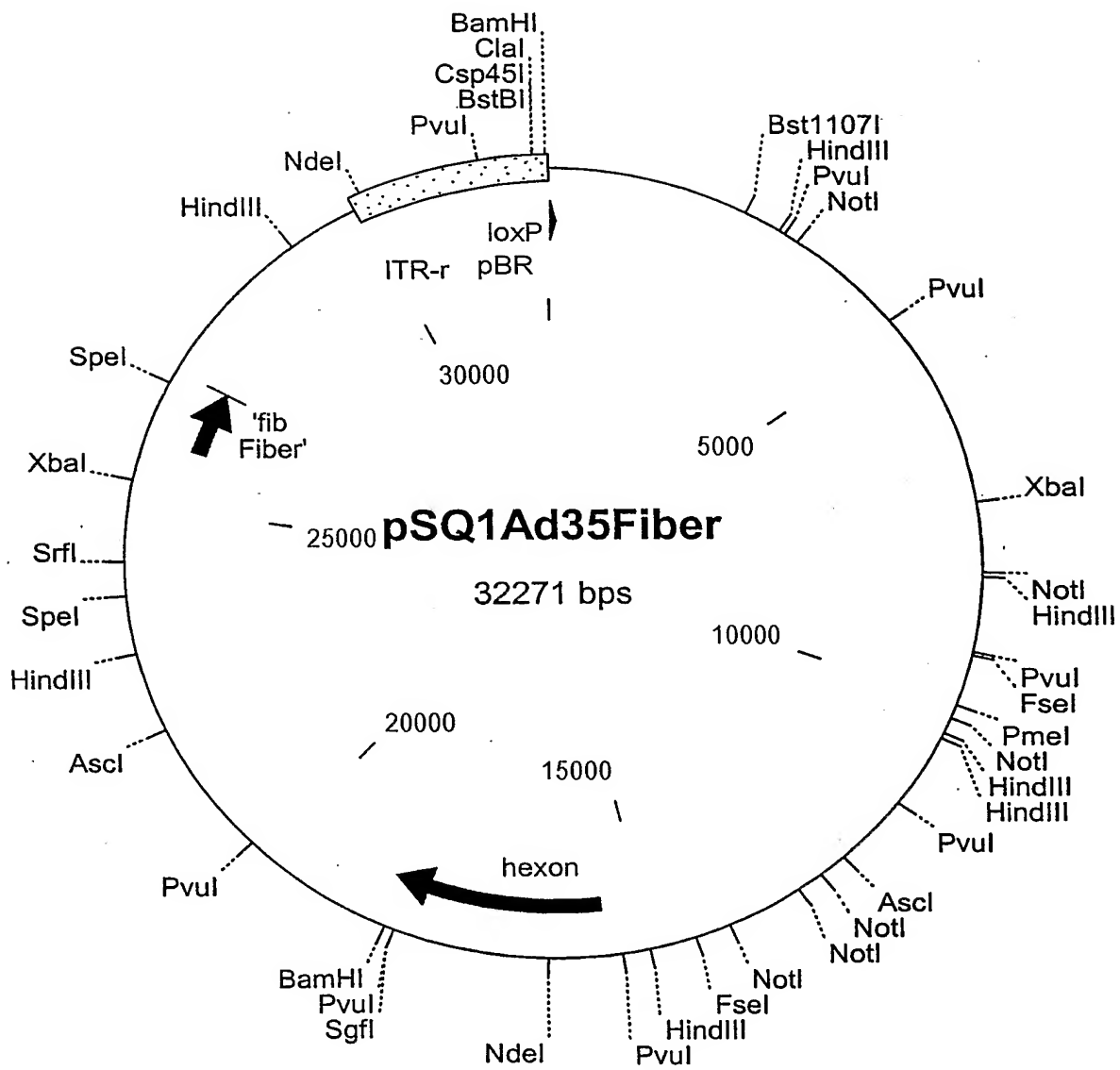
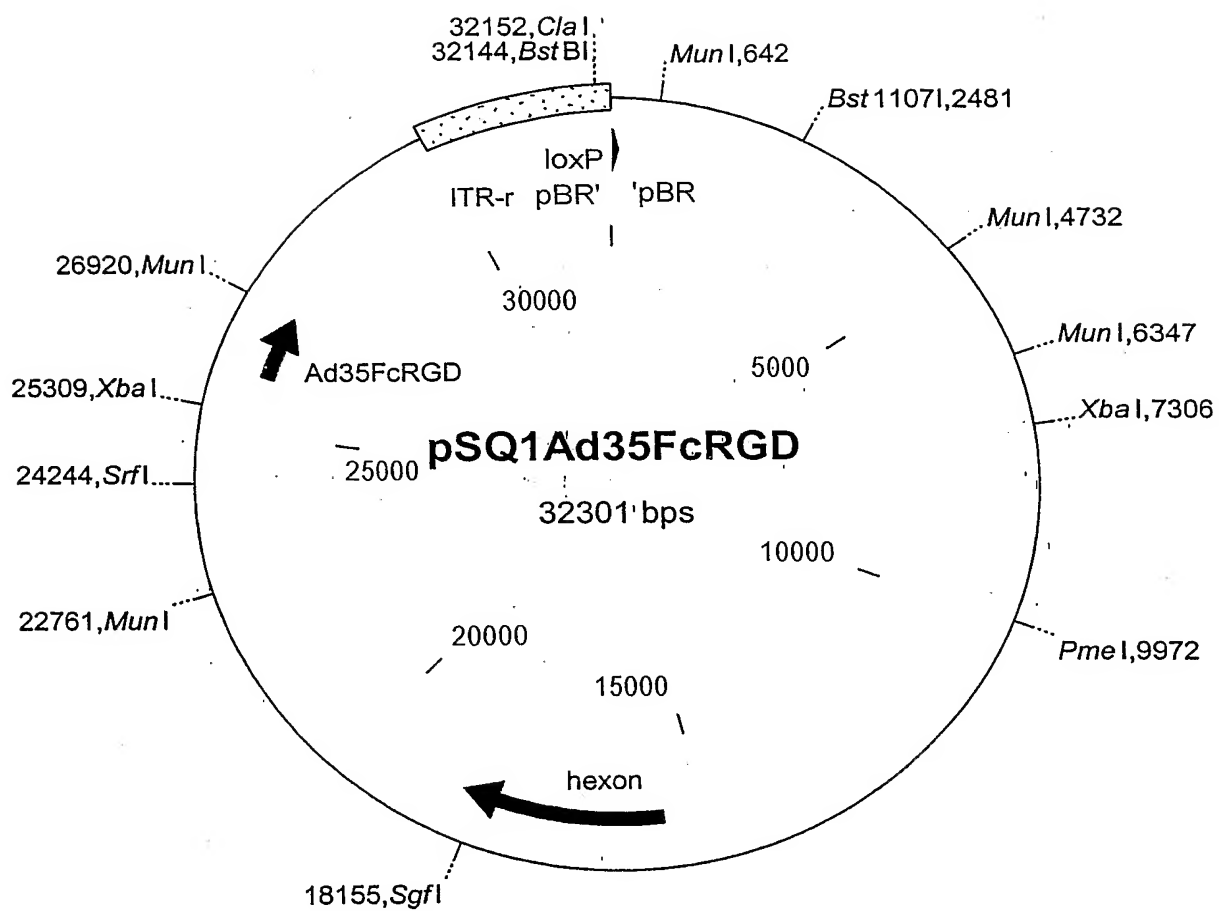


FIG. 16

22/35

**FIG. 17A**

23/35

**FIG. 17B**

24/35

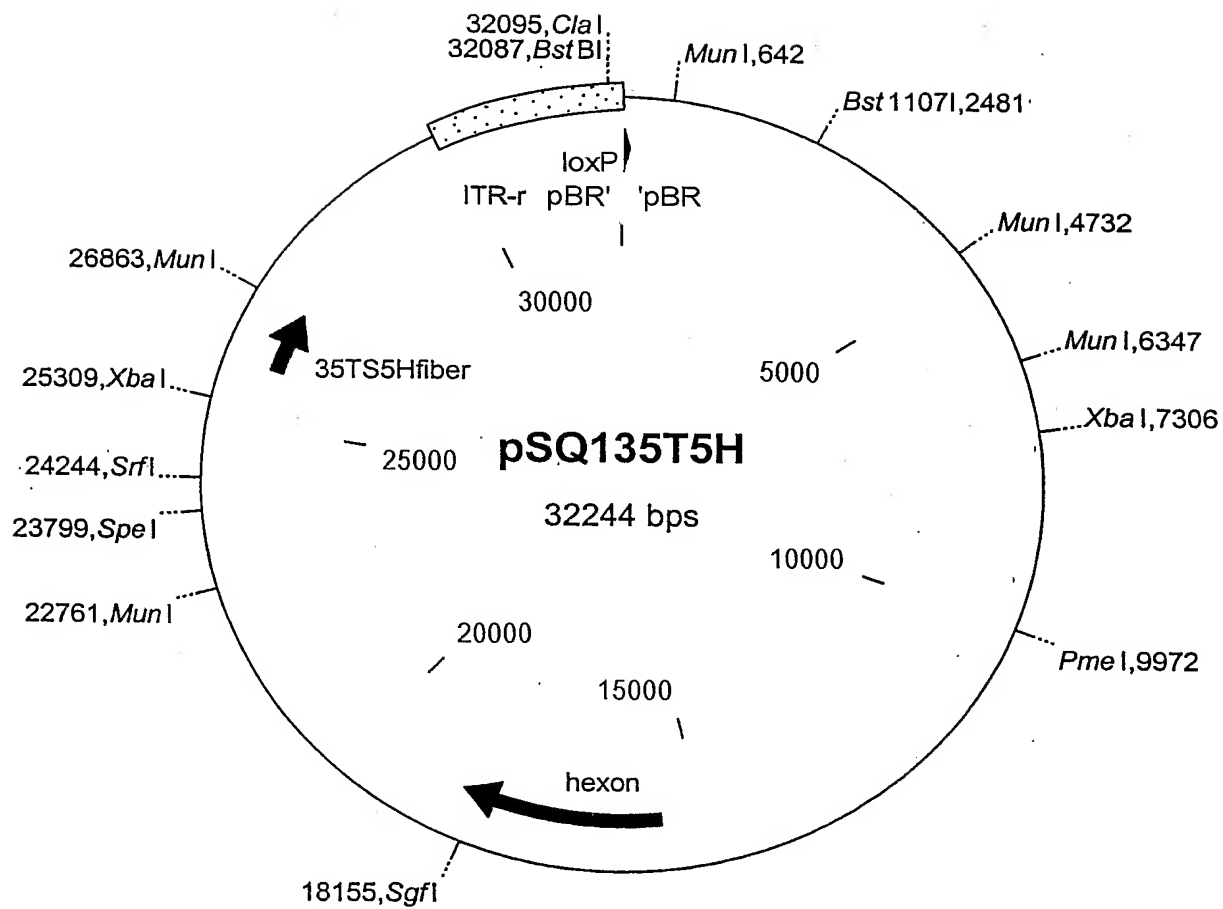


FIG. 18A

25/35

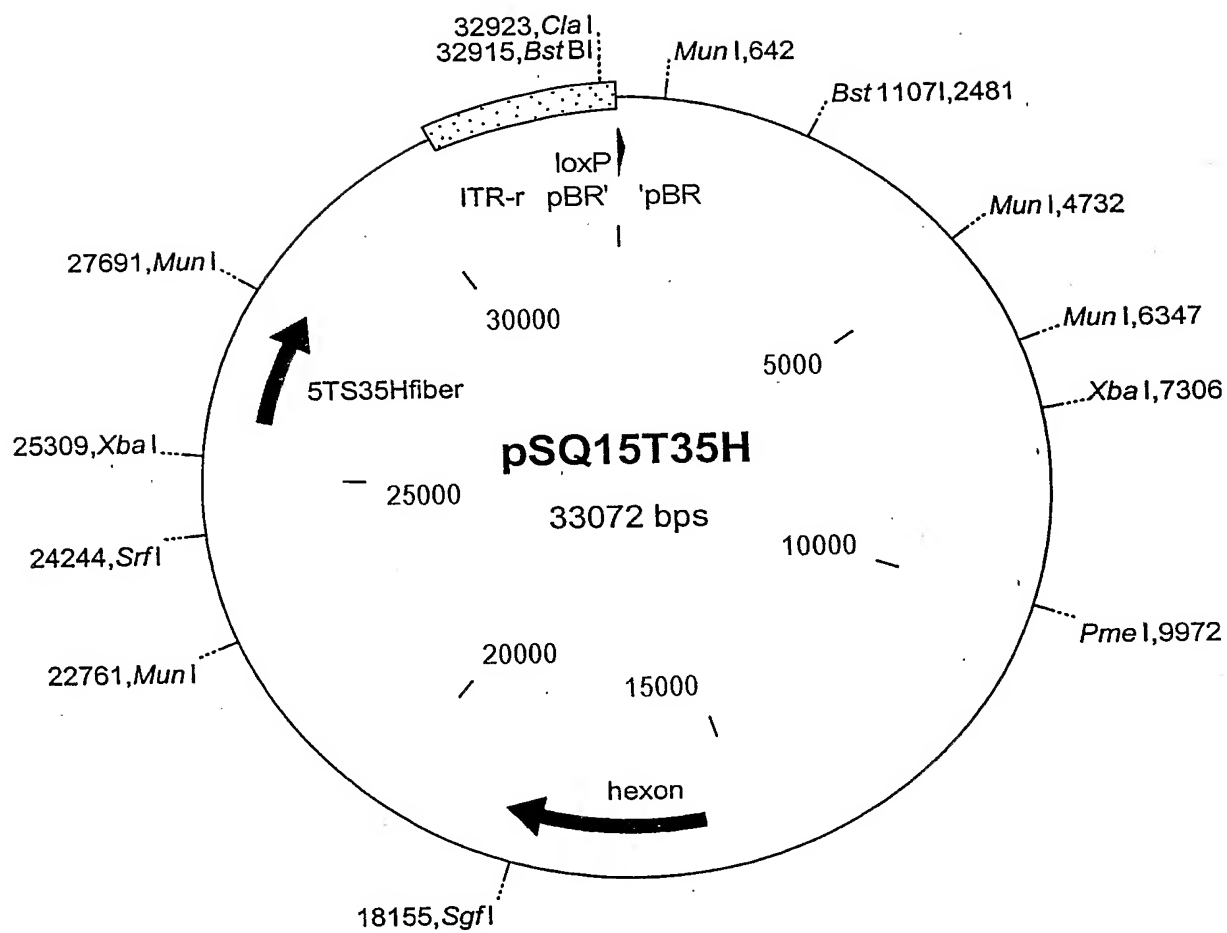
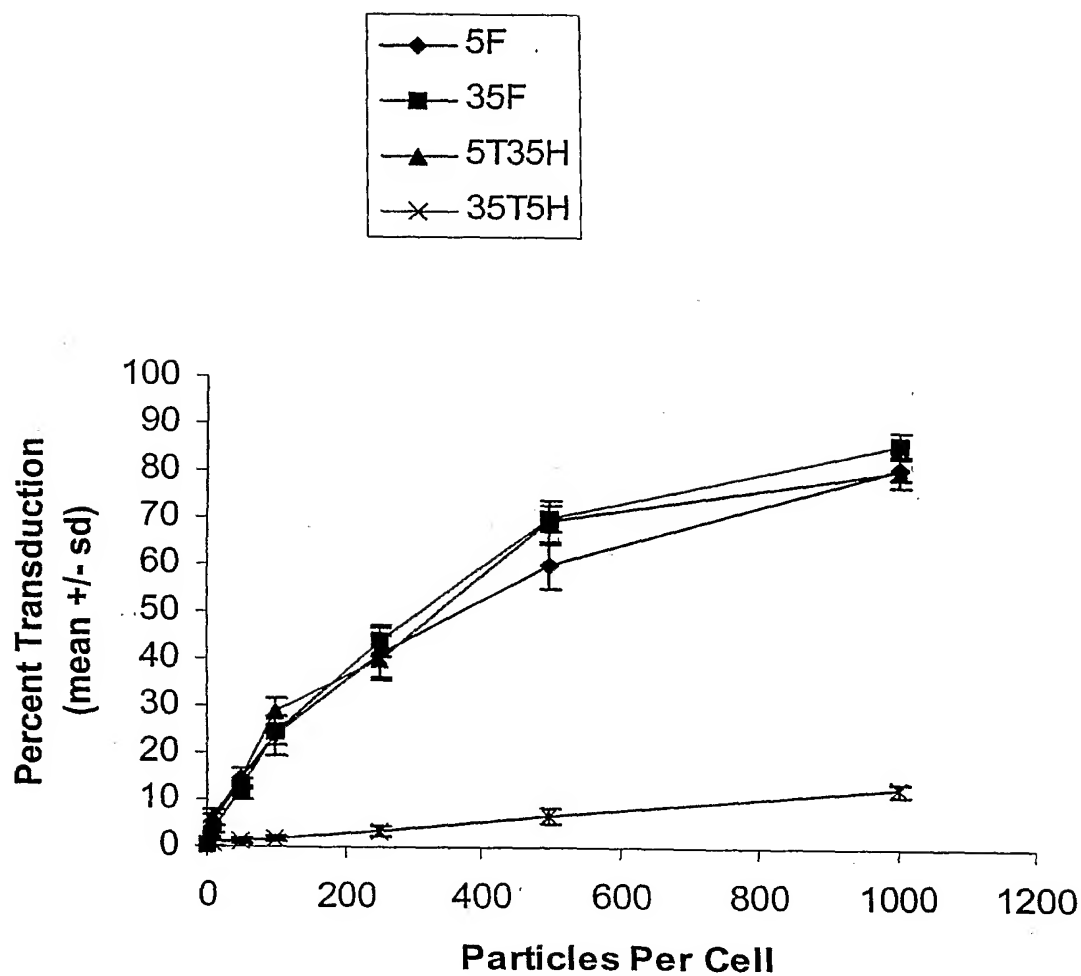
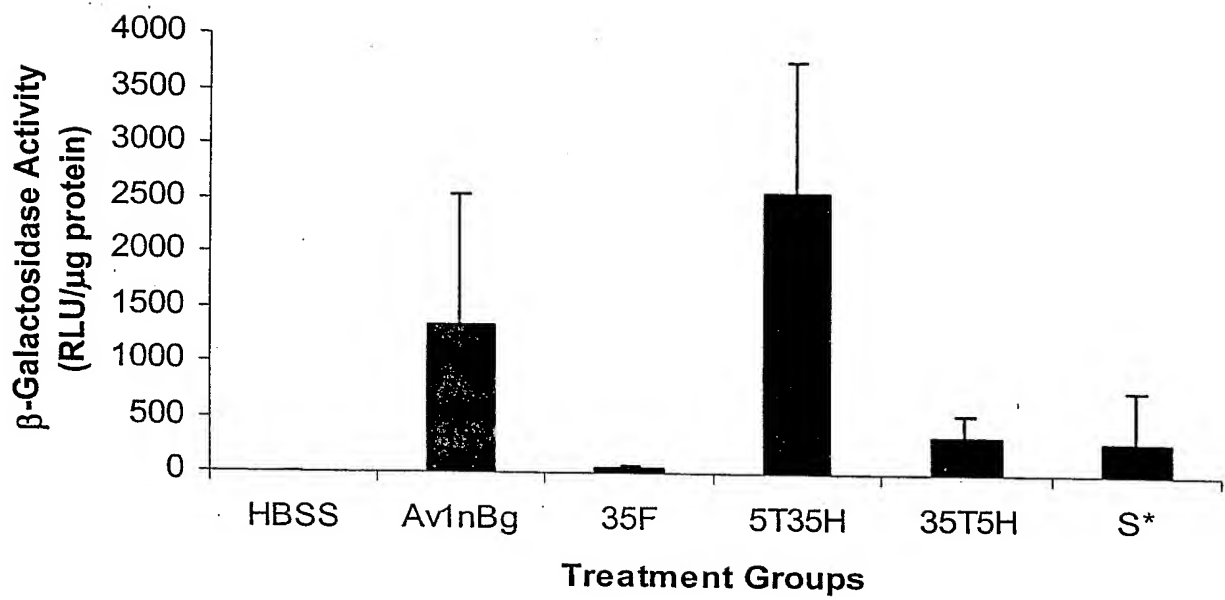


FIG. 18B

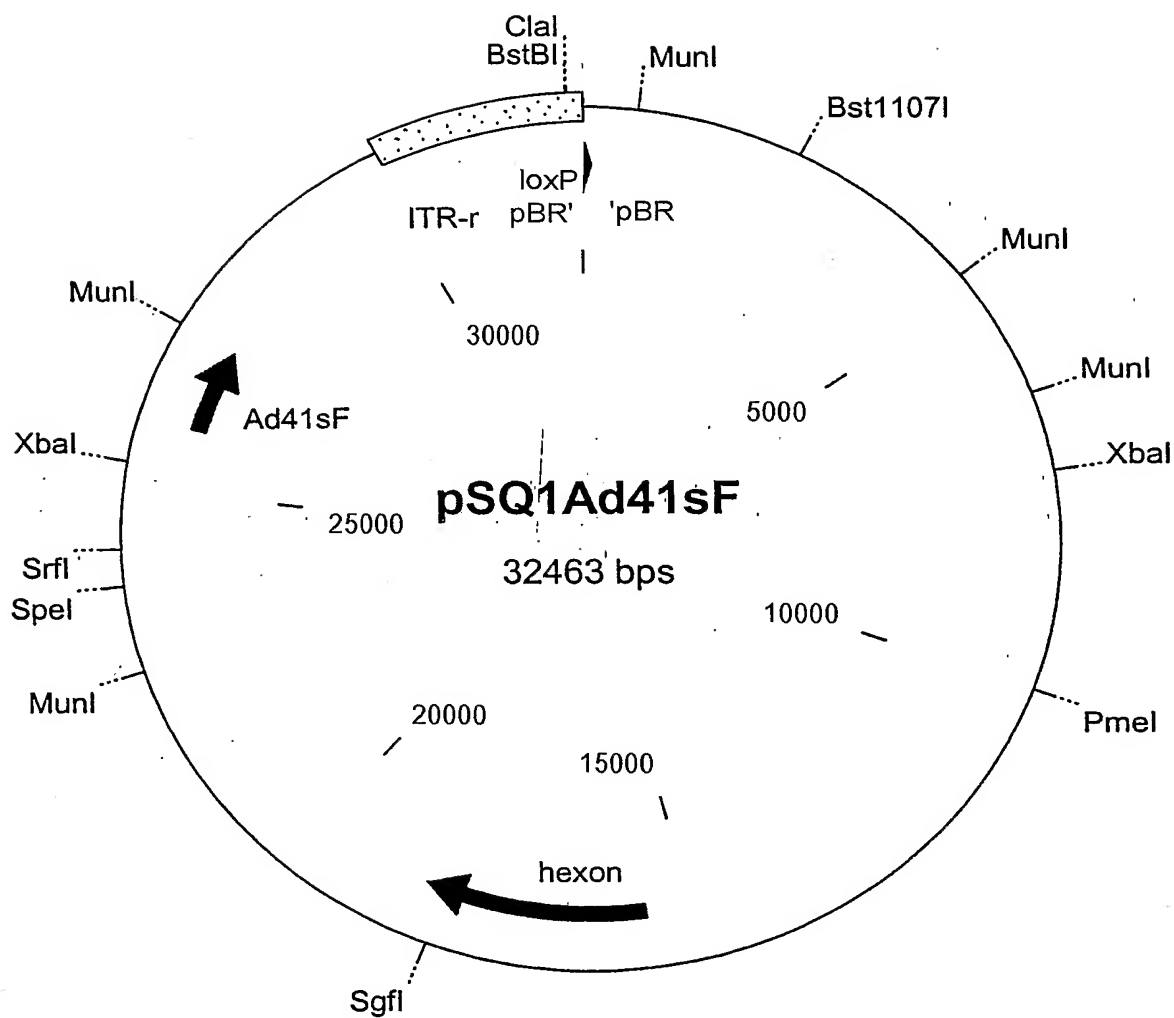
26/35

**FIG. 19**

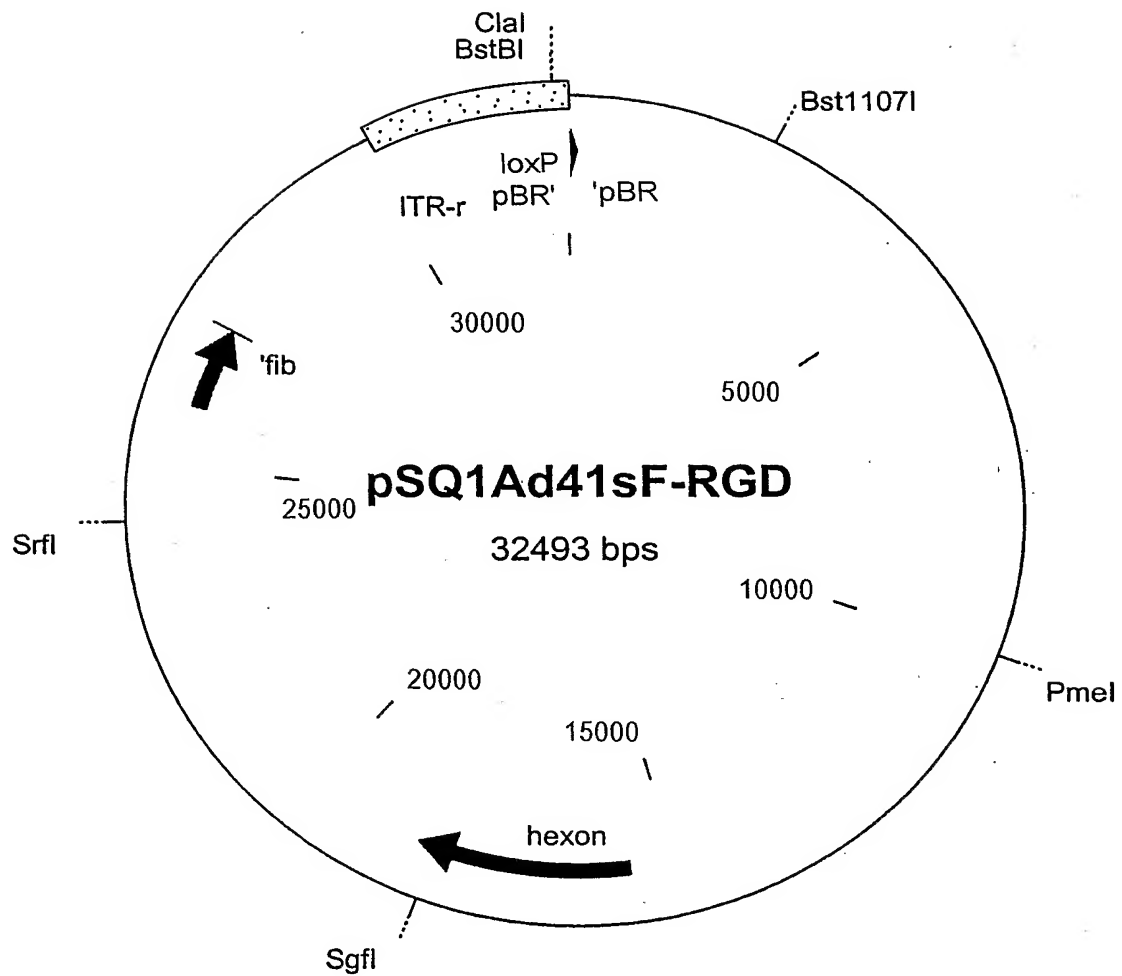
27/35

**FIG. 20**

28/35

**FIG. 21A**

29/35

**FIG. 21B**

30/35

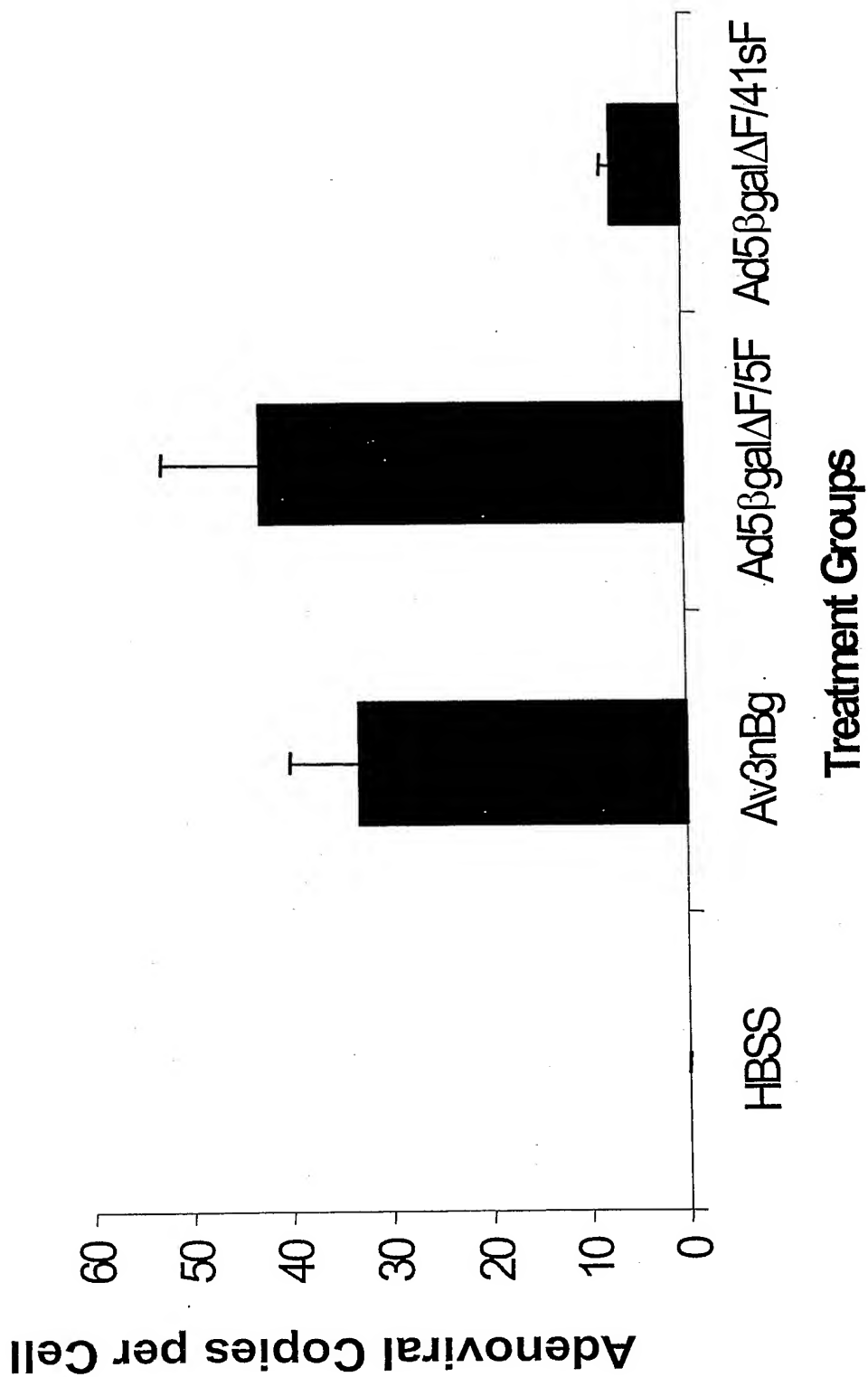


FIG. 22

31/35

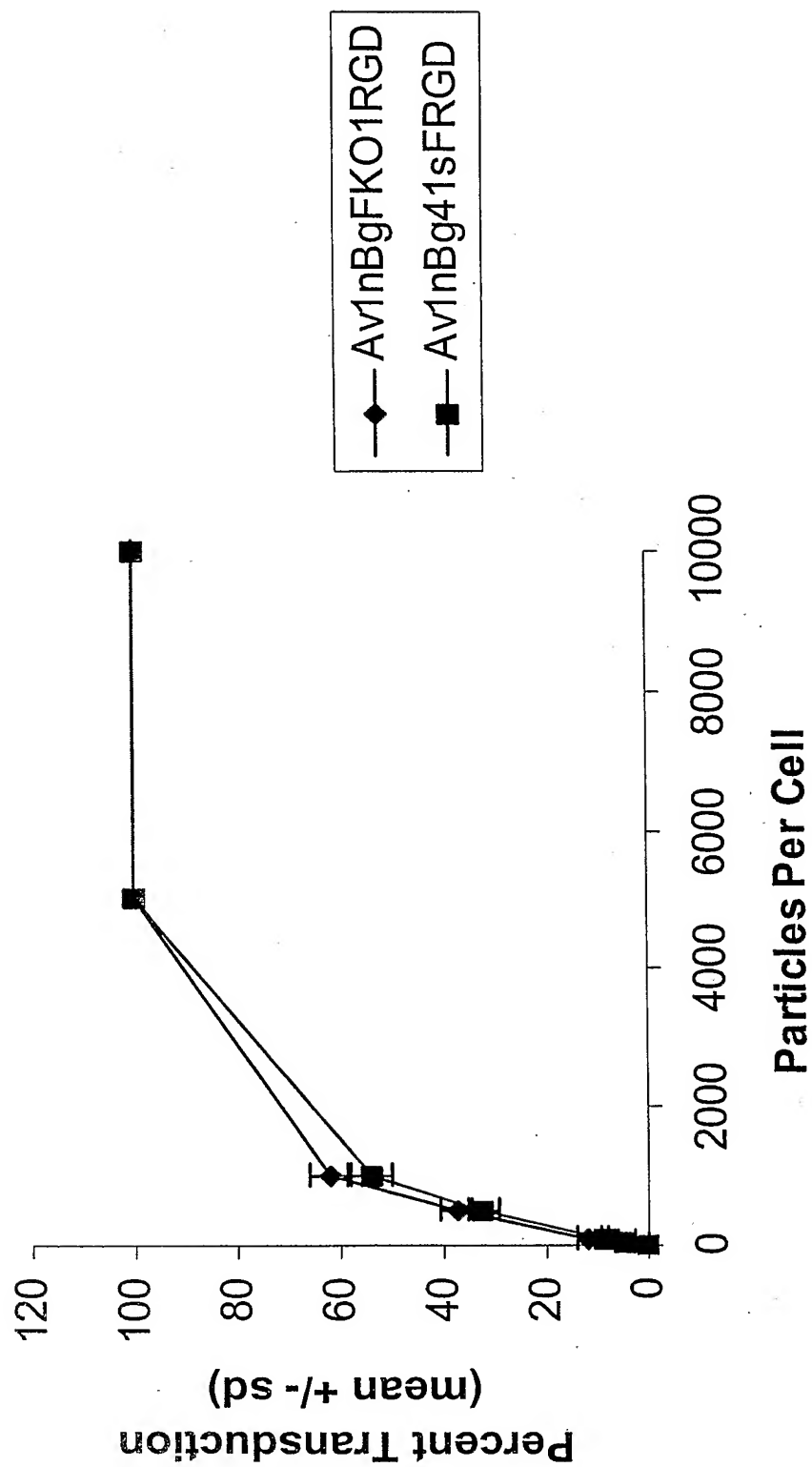


FIG. 23

32 / 35

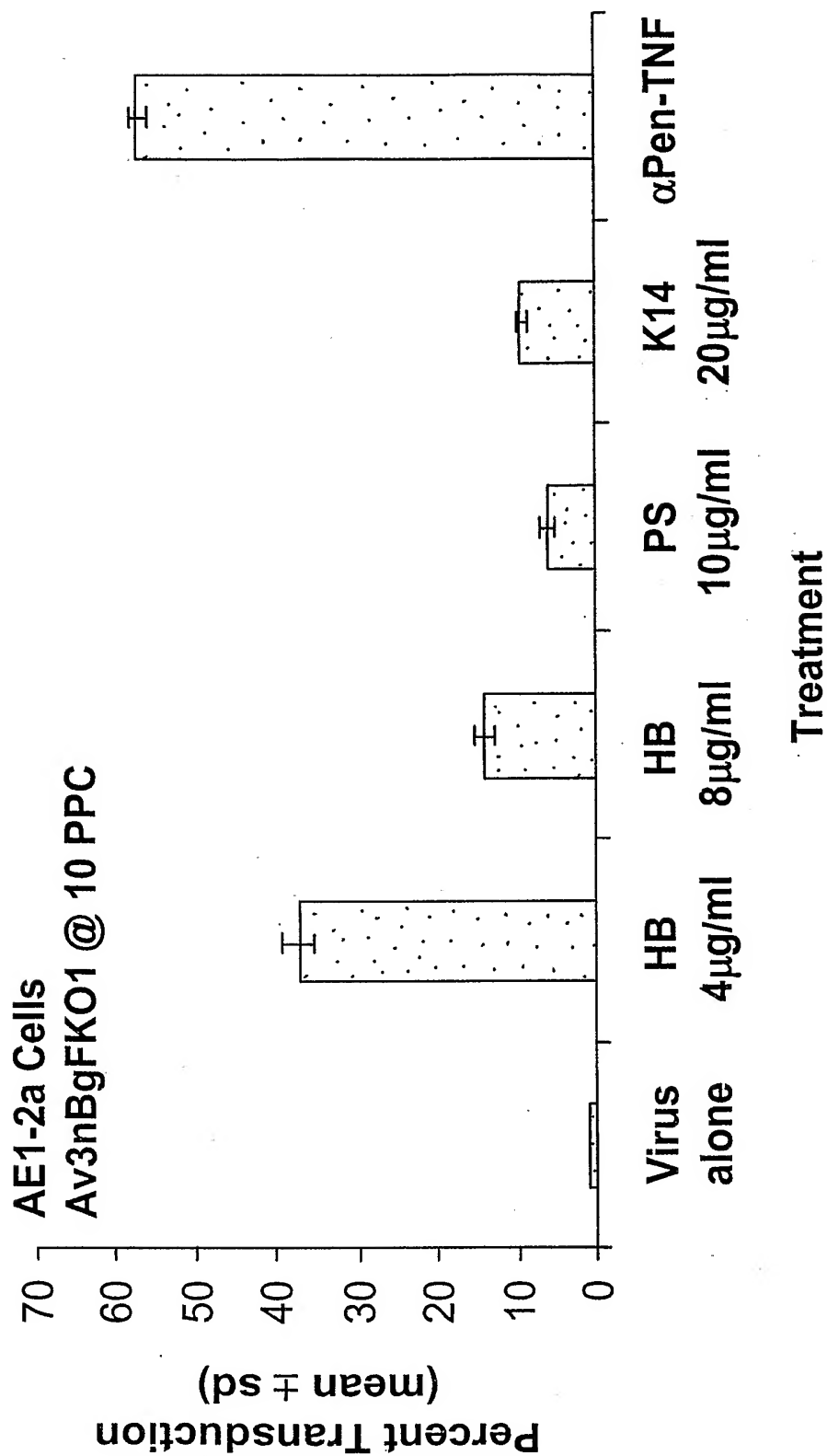


FIG. 24

33/35

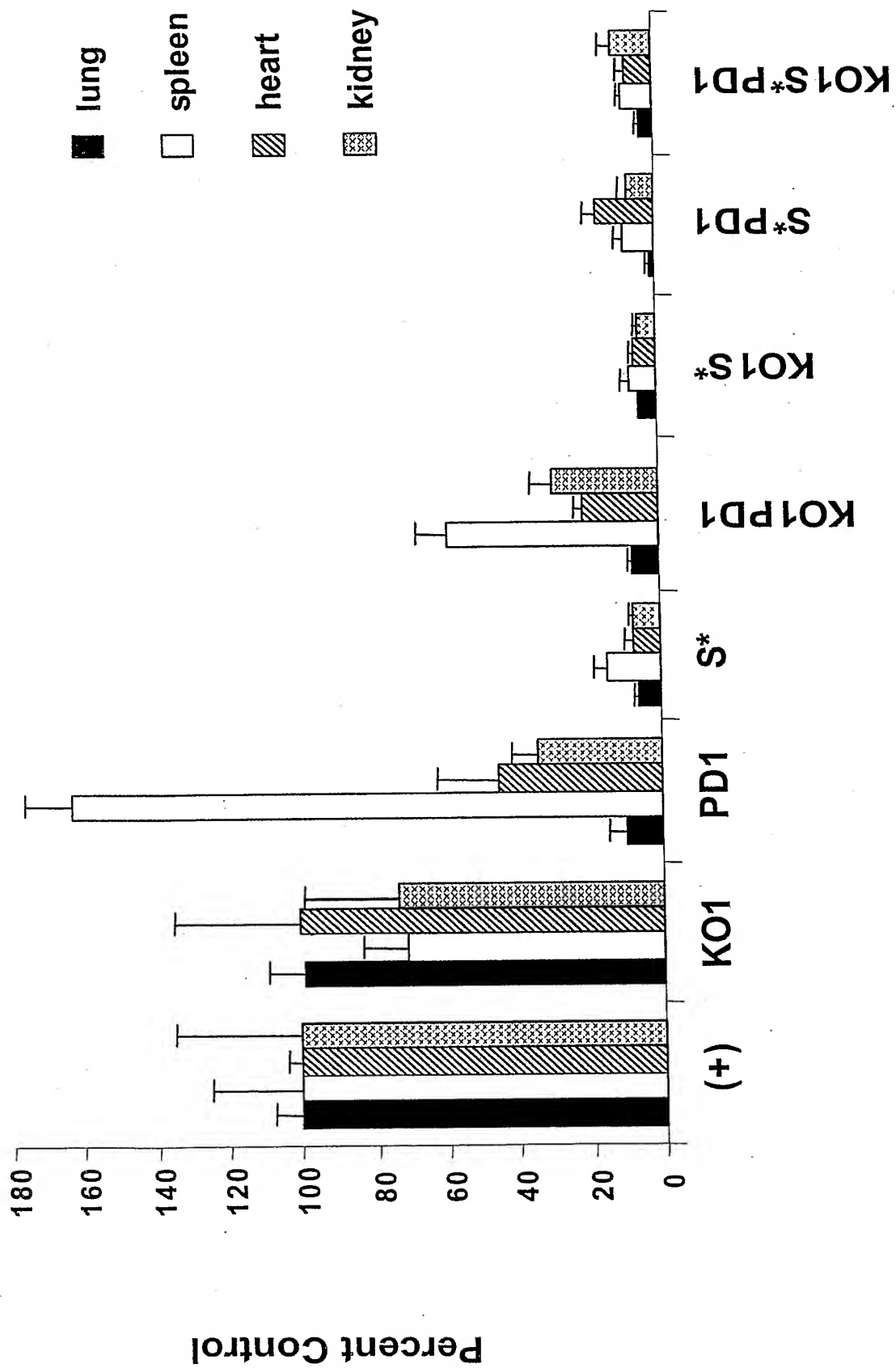


FIG. 25

34/35

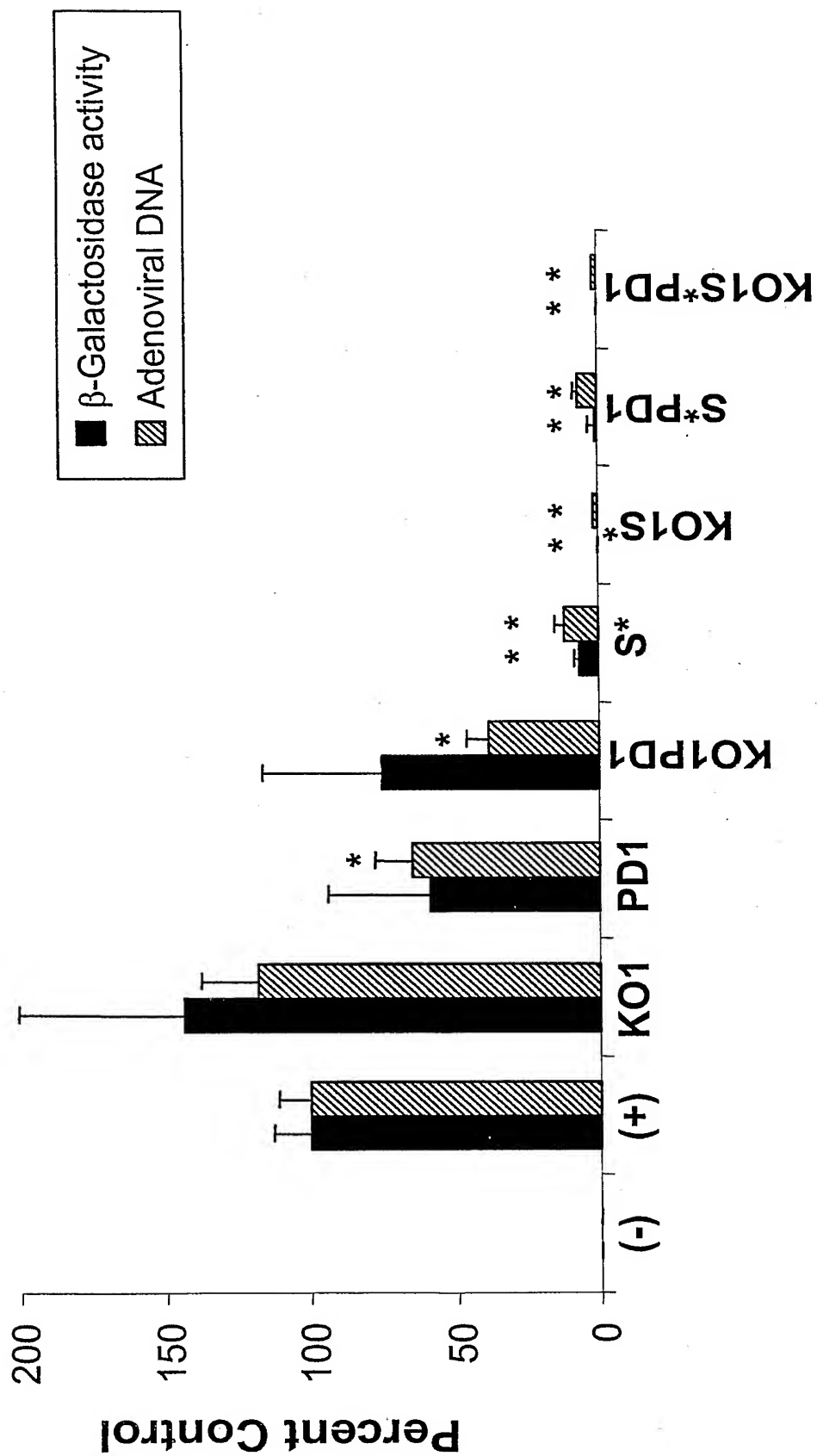


FIG. 26

35/35

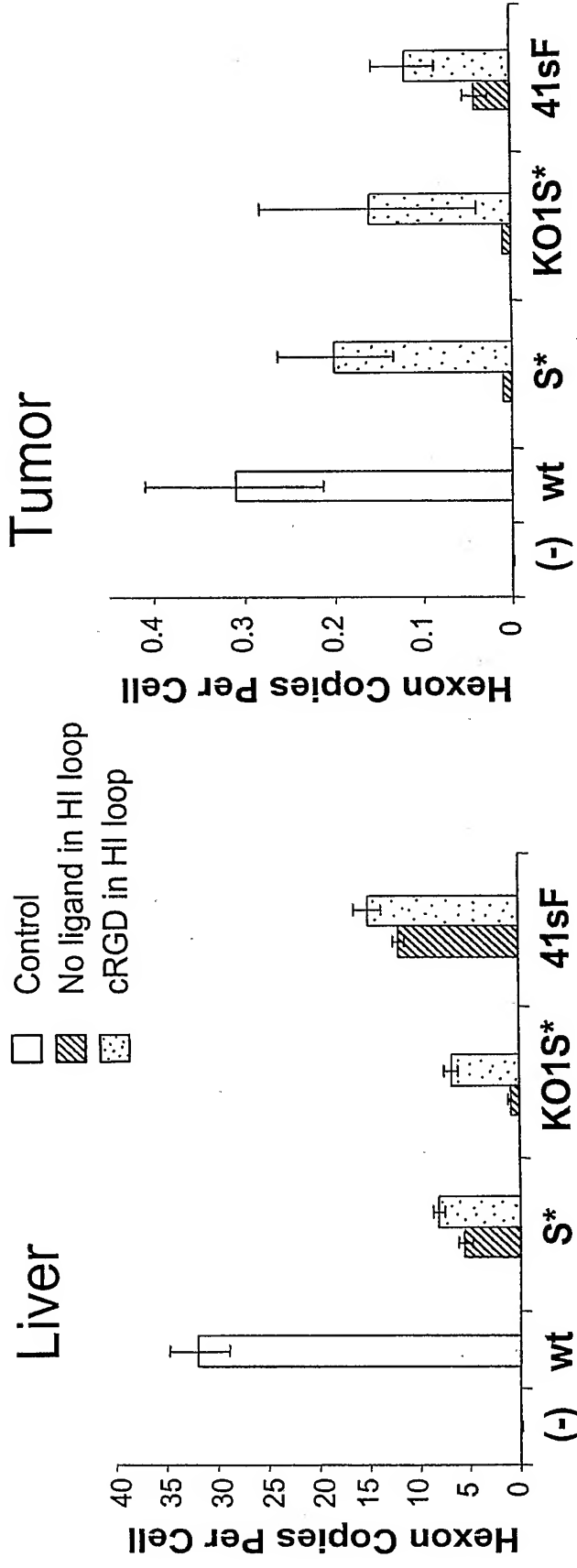


FIG. 27B

FIG. 27A

-1-

SEQUENCE LISTING

<110> The Scripps Research Institute
 Von Seggern, Daniel J.

<120> ADENOVIRUS PARTICLES WITH ENHANCED INFECTIVITY OF DENDRITIC CELLS AND
 PARTICLES WITH DECREASED INFECTIVITY OF HEPATOCYTES

<130> 22908-1239PC

<140> Not yet assigned

<141> Herewith

<150> US 60/459,000

<151> 2003-03-28

<150> US 60/467,500

<151> 2003-05-01

<160> 118

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1746

<212> DNA

<213> Adenovirus type 5

<220>

<221> CDS

<222> (1)...(1746)

<400> 1

atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca	48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro	
1 5 10 15	
tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc	96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro	
20 25 30	
ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct	144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser	
35 40 45	
ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc	192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu	
50 55 60	
aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc	240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser	
65 70 75 80	
caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac	288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn	
85 90 95	
ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta	336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu	
100 105 110	

-2-

act	gtg	gct	gcc	gcc	gca	cct	cta	atg	gtc	gcg	ggc	aac	aca	ctc	acc	384
Thr	Val	Ala	Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr	
		115					120					125				
atg	caa	tca	cag	gcc	ccg	cta	acc	gtg	cac	gac	tcc	aaa	ctt	agc	att	432
Met	Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp	Ser	Lys	Leu	Ser	Ile	
	130					135					140					
gcc	acc	caa	gga	ccc	ctc	aca	gtg	tca	gaa	gga	aag	cta	gcc	ctg	caa	480
Ala	Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln	
145					150					155					160	
aca	tca	ggc	ccc	ctc	acc	acc	acc	gat	agc	agt	acc	ctt	act	atc	act	528
Thr	Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr	
			165						170					175		
gcc	tca	ccc	cct	cta	act	act	gcc	act	ggg	agc	ttg	ggc	att	gac	ttg	576
Ala	Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu	
			180					185					190			
aaa	gag	ccc	att	tat	aca	caa	aat	gga	aaa	cta	gga	cta	aag	tac	ggg	624
Lys	Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly	
		195					200					205				
gct	cct	ttg	cat	gta	aca	gac	gac	cta	aac	act	ttg	acc	gta	gca	act	672
Ala	Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr	Leu	Thr	Val	Ala	Thr	
	210					215					220					
ggg	cca	ggg	gtg	act	att	aat	aat	act	tcc	ttg	caa	act	aaa	gtt	act	720
Gly	Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr	
225				230					235					240		
gga	gcc	ttg	ggg	ttt	gat	tca	caa	ggc	aat	atg	caa	ctt	aat	gta	gca	768
Gly	Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala	
				245					250					255		
gga	gga	cta	agg	att	gat	tct	caa	aac	aga	cgc	ctt	ata	ctt	gat	gtt	816
Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val	
			260					265					270			
agt	tat	ccg	ttt	gat	gct	caa	aac	caa	cta	aat	cta	aga	cta	gga	cag	864
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln	
		275					280					285				
ggc	cct	ctt	ttt	ata	aac	tca	gcc	cac	aac	ttg	gat	att	aac	tac	aac	912
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn	
	290					295					300					
aaa	ggc	ctt	tac	ttg	ttt	aca	gct	tca	aac	aat	tcc	aaa	aag	ctt	gag	960
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu	
305					310					315					320	
gtt	aac	cta	agc	act	gcc	aag	ggg	ttg	atg	ttt	gac	gct	aca	gcc	ata	1008
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile	
				325					330					335		
gcc	att	aat	gca	gga	gat	ggg	ctt	gaa	ttt	ggg	tca	cct	aat	gca	cca	1056
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro	
			340					345					350			
aac	aca	aat	ccc	ctc	aaa	aca	aaa	att	ggc	cat	ggc	cta	gaa	ttt	gat	1104

-3-

Asn Thr	Asn Pro	Leu Lys	Thr Lys	Ile Gly	His Gly	Leu Glu	Phe Asp	
	355		360			365		
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac	1152							
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp								
370	375	380						
agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act	1200							
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr								
385	390	395	400					
ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag	1248							
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu								
	405	410	415					
aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata	1296							
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile								
	420	425	430					
ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata	1344							
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile								
	435	440	445					
tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat	1392							
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn								
	450	455	460					
gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt	1440							
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe								
	465	470	475	480				
aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga	1488							
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly								
	485	490	495					
ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc	1536							
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala								
	500	505	510					
aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa	1584							
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys								
	515	520	525					
cct gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac	1632							
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp								
	530	535	540					
aca act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc	1680							
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly								
	545	550	555	560				
cac aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca	1728							
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser								
	565	570	575					
tac att gcc caa gaa taa	1746							
Tyr Ile Ala Gln Glu *								
	580							

-4-

<211> 580

<212> PRT

<213> Adenovirus type 5

<400> 2

Lys	Arg	Ala	Arg	Pro	Ser	Glu	Asp	Thr	Phe	Asn	Pro	Val	Tyr	Pro	Tyr
1				5					10					15	
Asp	Thr	Glu	Thr	Gly	Pro	Pro	Thr	Val	Pro	Phe	Leu	Thr	Pro	Pro	Phe
			20					25					30		
Val	Ser	Pro	Asn	Gly	Phe	Gln	Glu	Ser	Pro	Pro	Gly	Val	Leu	Ser	Leu
		35				40						45			
Arg	Leu	Ser	Glu	Pro	Leu	Val	Thr	Ser	Asn	Gly	Met	Leu	Ala	Leu	Lys
	50					55					60				
Met	Gly	Asn	Gly	Leu	Ser	Leu	Asp	Glu	Ala	Gly	Asn	Leu	Thr	Ser	Gln
65					70					75				80	
Asn	Val	Thr	Thr	Val	Ser	Pro	Pro	Leu	Lys	Lys	Thr	Lys	Ser	Asn	Ile
				85					90					95	
Asn	Leu	Glu	Ile	Ser	Ala	Pro	Leu	Thr	Val	Thr	Ser	Glu	Ala	Leu	Thr
			100					105					110		
Val	Ala	Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr	Met
		115					120					125			
Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp	Ser	Lys	Leu	Ser	Ile	Ala
	130					135					140				
Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln	Thr
145					150					155				160	
Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr	Ala
				165					170					175	
Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu	Lys
			180					185					190		
Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly	Ala
	195					200						205			
Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr	Leu	Thr	Val	Ala	Thr	Gly
	210				215						220				
Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr	Gly
225					230					235				240	
Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala	Gly
			245						250					255	
Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val	Ser
		260						265					270		
Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln	Gly
	275					280							285		
Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn	Lys
	290				295						300				
Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu	Val
305					310					315				320	
Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile	Ala
				325					330					335	
Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro	Asn
		340						345					350		
Thr	Asn	Pro	Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp	Ser
	355						360					365			
Asn	Lys	Ala	Met	Val	Pro	Lys	Leu	Gly	Thr	Gly	Leu	Ser	Phe	Asp	Ser
	370				375						380				
Thr	Gly	Ala	Ile	Thr	Val	Gly	Asn	Lys	Asn	Asn	Asp	Lys	Leu	Thr	Leu
385					390					395				400	
Trp	Thr	Thr	Pro	Ala	Pro	Ser	Pro	Asn	Cys	Arg	Leu	Asn	Ala	Glu	Lys
			405						410					415	
Asp	Ala	Lys	Leu	Thr	Leu	Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile	Leu
			420					425					430		
Ala	Thr	Val	Ser	Val	Leu	Ala	Val	Lys	Gly	Ser	Leu	Ala	Pro	Ile	Ser
		435					440						445		

[illegible]

<400>	3																
atg	aag	cg	gca	aga	ccg	tct	gaa	gat	acc	ttc	aac	ccc	gtg	tat	cca		48
Met	Lys	Arg	Ala	Arg	Pro	Ser	Glu	Asp	Thr	Phe	Asn	Pro	Val	Tyr	Pro		
1				5					10					15			
tat	gac	acg	gaa	acc	gg	cct	cca	act	gtg	cct	ttt	ctt	act	cct	ccc		96
Tyr	Asp	Thr	Glu	Thr	Gly	Pro	Pro	Thr	Val	Pro	Phe	Leu	Thr	Pro	Pro		
			20					25					30				
ttt	gta	tcc	ccc	aat	ggg	ttt	caa	gag	agt	ccc	cct	ggg	gta	ctc	tct		144
Phe	Val	Ser	Pro	Asn	Gly	Phe	Gln	Glu	Ser	Pro	Pro	Gly	Val	Leu	Ser		
		35					40					45					
ttg	cg	cta	tcc	gaa	cct	cta	gtt	acc	tcc	aat	ggc	atg	ctt	gcg	ctc		192
Leu	Arg	Leu	Ser	Glu	Pro	Leu	Val	Thr	Ser	Asn	Gly	Met	Leu	Ala	Leu		
	50					55					60						
aaa	atg	ggc	aac	ggc	ctc	tct	ctg	gac	gag	gcc	ggc	aac	ctt	acc	tcc		240
Lys	Met	Gly	Asn	Gly	Leu	Ser	Leu	Asp	Glu	Ala	Gly	Asn	Leu	Thr	Ser		
65				70					75					80			
caa	aat	gta	acc	act	gtg	agc	cca	cct	ctc	aaa	aaa	acc	aag	tca	aac		288
Gln	Asn	Val	Thr	Thr	Val	Ser	Pro	Pro	Leu	Lys	Lys	Thr	Lys	Ser	Asn		
				85					90					95			
ata	aac	ctg	gaa	ata	tct	gca	ccc	ctc	aca	gtt	acc	tca	gaa	gcc	cta		336
Ile	Asn	Leu	Glu	Ile	Ser	Ala	Pro	Leu	Thr	Val	Thr	Ser	Glu	Ala	Leu		
			100					105					110				
act	gtg	gct	gcc	gcc	gca	cct	cta	atg	gtc	gcg	ggc	aac	aca	ctc	acc		384

-6-

Thr	Val	Ala	Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr		
		115					120					125					
atg	caa	tca	cag	gcc	ccg	cta	acc	gtg	cac	gac	tcc	aaa	ctt	agc	att	432	
Met	Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp	Ser	Lys	Leu	Ser	Ile		
	130					135					140						
gcc	acc	caa	gga	ccc	ctc	aca	gtg	tca	gaa	gga	aag	cta	gcc	ctg	caa	480	
Ala	Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln		
145					150					155					160		
aca	tca	ggc	ccc	ctc	acc	acc	acc	gat	agc	agt	acc	ctt	act	atc	act	528	
Thr	Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr		
				165					170					175			
gcc	tca	ccc	cct	cta	act	act	gcc	act	ggg	agc	ttg	ggc	att	gac	ttg	576	
Ala	Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu		
			180					185					190				
aaa	gag	ccc	att	tat	aca	caa	aat	gga	aaa	cta	gga	cta	aag	tac	ggg	624	
Lys	Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly		
		195					200					205					
gct	cct	ttg	cat	gta	aca	gac	gac	cta	aac	act	ttg	acc	gta	gca	act	672	
Ala	Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr	Leu	Thr	Val	Ala	Thr		
	210					215					220						
ggg	cca	ggg	gtg	act	att	aat	aat	act	tcc	ttg	caa	act	aaa	gtt	act	720	
Gly	Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr		
225				230						235				240			
gga	gcc	ttg	ggg	ttt	gat	tca	caa	ggc	aat	atg	caa	ctt	aat	gta	gca	768	
Gly	Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala		
				245					250					255			
gga	gga	cta	agg	att	gat	tct	caa	aac	aga	cgc	ctt	ata	ctt	gat	gtt	816	
Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val		
			260					265					270				
agt	tat	ccg	ttt	gat	gct	caa	aac	caa	cta	aat	cta	aga	cta	gga	cag	864	
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln		
		275					280					285					
ggc	cct	ctt	ttt	ata	aac	tca	gcc	cac	aac	ttg	gat	att	aac	tac	aac	912	
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn		
	290					295					300						
aaa	ggc	ctt	tac	ttg	ttt	aca	gct	tca	aac	aat	tcc	aaa	aag	ctt	gag	960	
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu		
305				310					315					320			
gtt	aac	cta	agc	act	gcc	aag	ggg	ttg	atg	ttt	gac	gct	aca	gcc	ata	1008	
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile		
				325					330					335			
gcc	att	aat	gca	gga	gat	ggg	ctt	gaa	ttt	ggg	tca	cct	aat	gca	cca	1056	
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro		
			340					345					350				
aac	aca	aat	ccc	ctc	aaa	aca	aaa	att	ggc	cat	ggc	cta	gaa	ttt	gat	1104	
Asn	Thr	Asn	Pro	Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp		

-7-

355	360	365	
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp 370 375 380			1152
agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr 385 390 395 400			1200
ttg tgg acc aca cca gct cca gag gct aac tgt aga cta aat gca gag Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu 405 410 415			1248
aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile 420 425 430			1296
ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile 435 440 445			1344
tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn 450 455 460			1392
gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe 465 470 475 480			1440
aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly 485 490 495			1488
ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala 500 505 510			1536
aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys 515 520 525			1584
cct gta aca cta acc att aca cta aac ggt aca cag gaa aca gga gac Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp 530 535 540			1632
aca act cca agt gca tac tct atg tca ttt tca tgg gac tgg tct ggc Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly 545 550 555 560			1680
cac aac tac att aat gaa ata ttt gcc aca tcc tct tac act ttt tca His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser 565 570 575			1728
tac att gcc caa gaa taa Tyr Ile Ala Gln Glu *			1746
580			

<210> 4

<211> 581

-8-

<212> PRT

<213> Artificial Sequence

<220>

<223> 5F KO1

<400> 4

Met	Lys	Arg	Ala	Arg	Pro	Ser	Glu	Asp	Thr	Phe	Asn	Pro	Val	Tyr	Pro
1				5					10					15	
Tyr	Asp	Thr	Glu	Thr	Gly	Pro	Pro	Thr	Val	Pro	Phe	Leu	Thr	Pro	Pro
			20					25					30		
Phe	Val	Ser	Pro	Asn	Gly	Phe	Gln	Glu	Ser	Pro	Pro	Gly	Val	Leu	Ser
		35				40						45			
Leu	Arg	Leu	Ser	Glu	Pro	Leu	Val	Thr	Ser	Asn	Gly	Met	Leu	Ala	Leu
	50					55					60				
Lys	Met	Gly	Asn	Gly	Leu	Ser	Leu	Asp	Glu	Ala	Gly	Asn	Leu	Thr	Ser
65					70				75						80
Gln	Asn	Val	Thr	Thr	Val	Ser	Pro	Pro	Leu	Lys	Lys	Thr	Lys	Ser	Asn
			85						90					95	
Ile	Asn	Leu	Glu	Ile	Ser	Ala	Pro	Leu	Thr	Val	Thr	Ser	Glu	Ala	Leu
			100					105					110		
Thr	Val	Ala	Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr
		115					120					125			
Met	Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp	Ser	Lys	Leu	Ser	Ile
	130					135					140				
Ala	Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln
145					150					155					160
Thr	Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr
			165					170						175	
Ala	Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu
			180					185					190		
Lys	Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly
		195				200					205				
Ala	Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr	Leu	Thr	Val	Ala	Thr
	210					215					220				
Gly	Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr
225					230					235					240
Gly	Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala
			245					250						255	
Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val
		260						265					270		
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln
		275					280					285			
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn
	290					295					300				
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu
305					310					315					320
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile
			325						330					335	
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro
			340					345					350		
Asn	Thr	Asn	Pro	Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp
		355					360					365			
Ser	Asn	Lys	Ala	Met	Val	Pro	Lys	Leu	Gly	Thr	Gly	Leu	Ser	Phe	Asp
	370					375					380				
Ser	Thr	Gly	Ala	Ile	Thr	Val	Gly	Asn	Lys	Asn	Asn	Asp	Lys	Leu	Thr
385					390					395					400
Leu	Trp	Thr	Thr	Pro	Ala	Pro	Glu	Ala	Asn	Cys	Arg	Leu	Asn	Ala	Glu
			405					410						415	
Lys	Asp	Ala	Lys	Leu	Thr	Leu	Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile
			420					425					430		

-9-

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
 435 440 445
 Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
 450 455 460
 Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
 465 470 475 480
 Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
 485 490 495
 Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
 500 505 510
 Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
 515 520 525
 Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
 530 535 540
 Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
 545 550 555 560
 His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
 565 570 575
 Tyr Ile Ala Gln Glu
 580

<210> 5
 <211> 1776
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5F KO1RGD
 <221> CDS
 <222> (1)...(1746)

<400> 5
 atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
 35 40 45
 ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
 85 90 95
 ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
 100 105 110

-10-

act	gtg	gct	gcc	gcc	gca	cct	cta	atg	gtc	gcg	ggc	aac	aca	ctc	acc	384
Thr	Val		Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr	
		115					120					125				
atg	caa	tca	cag	gcc	ccg	cta	acc	gtg	cac	gac	tcc	aaa	ctt	agc	att	432
Met	Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp		Lys	Leu	Ser	Ile	
	130					135					140					
gcc	acc	caa	gga	ccc	ctc	aca	gtg	tca	gaa	gga	aag	cta	gcc	ctg	caa	480
Ala	Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln	
145					150					155					160	
aca	tca	ggc	ccc	ctc	acc	acc	acc	gat	agc	agt	acc	ctt	act	atc	act	528
Thr	Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr	
			165						170					175		
gcc	tca	ccc	cct	cta	act	act	gcc	act	ggg	agc	ttg	ggc	att	gac	ttg	576
Ala	Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu	
			180					185					190			
aaa	gag	ccc	att	tat	aca	caa	aat	gga	aaa	cta	gga	cta	aag	tac	ggg	624
Lys	Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly	
		195					200					205				
gct	cct	ttg	cat	gta	aca	gac	gac	cta	aac	act	ttg	acc	gta	gca	act	672
Ala	Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr		Leu	Thr	Val	Ala	Thr
	210					215						220				
ggg	cca	ggg	gtg	act	att	aat	aat	act	tcc	ttg	caa	act	aaa	gtt	act	720
Gly	Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr	
225				230						235					240	
gga	gcc	ttg	ggg	ttt	gat	tca	caa	ggc	aat	atg	caa	ctt	aat	gta	gca	768
Gly	Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala	
				245					250					255		
gga	gga	cta	agg	att	gat	tct	caa	aac	aga	cgc	ctt	ata	ctt	gat	gtt	816
Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val	
			260					265					270			
agt	tat	ccg	ttt	gat	gct	caa	aac	caa	cta	aat	cta	aga	cta	gga	cag	864
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln	
		275					280					285				
ggc	cct	ctt	ttt	ata	aac	tca	gcc	cac	aac	ttg	gat	att	aac	tac	aac	912
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn	
	290					295					300					
aaa	ggc	ctt	tac	ttg	ttt	aca	gct	tca	aac	aat	tcc	aaa	aag	ctt	gag	960
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu	
305					310					315					320	
gtt	aac	cta	agc	act	gcc	aag	ggg	ttg	atg	ttt	gac	gct	aca	gcc	ata	1008
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile	
				325					330					335		
gcc	att	aat	gca	gga	gat	ggg	ctt	gaa	ttt	ggg	tca	cct	aat	gca	cca	1056
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro	
			340					345					350			

-11-

aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat	1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp	
355 360 365	
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac	1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp	
370 375 380	
agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act	1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr	
385 390 395 400	
ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag	1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu	
405 410 415	
aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata	1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile	
420 425 430	
ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata	1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile	
435 440 445	
tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat	1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn	
450 455 460	
gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt	1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe	
465 470 475 480	
aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga	1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly	
485 490 495	
ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc	1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala	
500 505 510	
aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa	1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys	
515 520 525	
cct gta aca cta acc att aca cta aac ggt aca cag gaa aca ggt gat	1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp	
530 535 540	
cat tgt gat tgt cgt ggt gat tgt ttt tgt aca act cca agt gca tac	1680
His Cys Asp Cys Arg Gly Asp Cys Phe Cys Thr Thr Pro Ser Ala Tyr	
545 550 555 560	
tct atg tca ttt tca tgg gac tgg tct ggc cac aac tac att aat gaa	1728
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu	
565 570 575	
ata ttt gcc aca tcc tct tacacttttt catacattgc ccaagaataa	1776
Ile Phe Ala Thr Ser Ser	
580	

-12-

<210> 6
 <211> 582
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5F KO1RGD

<400> 6
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
 35 40 45
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
 85 90 95
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
 100 105 110
 Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
 115 120 125
 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
 130 135 140
 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
 145 150 155 160
 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
 165 170 175
 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
 180 185 190
 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
 195 200 205
 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
 210 215 220
 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
 225 230 235 240
 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
 245 250 255
 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
 260 265 270
 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
 275 280 285
 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
 290 295 300
 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
 305 310 315 320
 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
 325 330 335
 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
 340 345 350
 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
 355 360 365
 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
 370 375 380
 Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
 385 390 395 400
 Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
 405 410 415

[illegible]

```
<220>  
<223> 5F KO12  
  
<221> CDS  
<222> (1) ... (1746)
```

<400> 7	atg	aag	cgc	gca	aga	ccg	tct	gaa	gat	acc	ttc	aac	ccc	gtg	tat	cca	48
Met	Lys	Arg	Ala	Arg	Pro	Ser	Glu	Asp	Thr	Phe	Asn	Pro	Val	Tyr	Pro		
1				5					10					15			
tat	gac	acg	gaa	acc	ggg	cct	cca	act	gtg	cct	ttt	ctt	act	cct	ccc	96	
Tyr	Asp	Thr	Glu	Thr	Gly	Pro	Pro	Thr	Val	Pro	Phe	Leu	Thr	Pro	Pro		
			20					25					30				
ttt	gta	tcc	ccc	aat	ggg	ttt	caa	gag	agt	ccc	cct	ggg	gta	ctc	tct	144	
Phe	Val	Ser	Pro	Asn	Gly	Phe	Gln	Glu	Ser	Pro	Pro	Gly	Val	Leu	Ser		
		35					40					45					
ttg	cgc	cta	tcc	gaa	cct	cta	gtt	acc	tcc	aat	ggc	atg	ctt	gcg	ctc	192	
Leu	Arg	Leu	Ser	Glu	Pro	Leu	Val	Thr	Ser	Asn	Gly	Met	Leu	Ala	Leu		
	50					55					60						
aaa	atg	ggc	aac	ggc	ctc	tct	ctg	gac	gag	gcc	ggc	aac	ctt	acc	tcc	240	
Lys	Met	Gly	Asn	Gly	Leu	Ser	Leu	Asp	Glu	Ala	Gly	Asn	Leu	Thr	Ser		
65				70					75					80			
caa	aat	gta	acc	act	gtg	agc	cca	cct	ctc	aaa	aaa	acc	aag	tca	aac	288	
Gln	Asn	Val	Thr	Thr	Val	Ser	Pro	Pro	Leu	Lys	Lys	Thr	Lys	Ser	Asn		
				85					90					95			
ata	aac	ctg	gaa	ata	tct	gca	ccc	ctc	aca	gtt	acc	tca	gaa	gcc	cta	336	

-14-

Ile	Asn	Leu	Glu	Ile	Ser	Ala	Pro	Leu	Thr	Val	Thr	Ser	Glu	Ala	Leu	
			100					105					110			
act	gtg	gct	gcc	gcc	gca	cct	cta	atg	gtc	gcg	ggc	aac	aca	ctc	acc	384
Thr	Val	Ala	Ala	Ala	Ala	Pro	Leu	Met	Val	Ala	Gly	Asn	Thr	Leu	Thr	
		115					120					125				
atg	caa	tca	cag	gcc	ccg	cta	acc	gtg	cac	gac	tcc	aaa	ctt	agc	att	432
Met	Gln	Ser	Gln	Ala	Pro	Leu	Thr	Val	His	Asp	Ser	Lys	Leu	Ser	Ile	
	130					135					140					
gcc	acc	caa	gga	ccc	ctc	aca	gtg	tca	gaa	gga	aag	cta	gcc	ctg	caa	480
Ala	Thr	Gln	Gly	Pro	Leu	Thr	Val	Ser	Glu	Gly	Lys	Leu	Ala	Leu	Gln	
145					150					155					160	
aca	tca	ggc	ccc	ctc	acc	acc	acc	gat	agc	agt	acc	ctt	act	atc	act	528
Thr	Ser	Gly	Pro	Leu	Thr	Thr	Thr	Asp	Ser	Ser	Thr	Leu	Thr	Ile	Thr	
				165					170					175		
gcc	tca	ccc	cct	cta	act	act	gcc	act	ggg	agc	ttg	ggc	att	gac	ttg	576
Ala	Ser	Pro	Pro	Leu	Thr	Thr	Ala	Thr	Gly	Ser	Leu	Gly	Ile	Asp	Leu	
			180					185					190			
aaa	gag	ccc	att	tat	aca	caa	aat	gga	aaa	cta	gga	cta	aag	tac	ggg	624
Lys	Glu	Pro	Ile	Tyr	Thr	Gln	Asn	Gly	Lys	Leu	Gly	Leu	Lys	Tyr	Gly	
		195					200					205				
gct	cct	ttg	cat	gta	aca	gac	gac	cta	aac	act	ttg	acc	gta	gca	act	672
Ala	Pro	Leu	His	Val	Thr	Asp	Asp	Leu	Asn	Thr	Leu	Thr	Val	Ala	Thr	
	210					215					220					
ggg	cca	ggg	gtg	act	att	aat	aat	act	tcc	ttg	caa	act	aaa	gtt	act	720
Gly	Pro	Gly	Val	Thr	Ile	Asn	Asn	Thr	Ser	Leu	Gln	Thr	Lys	Val	Thr	
225				230					235					240		
gga	gcc	ttg	ggg	ttt	gat	tca	caa	ggc	aat	atg	caa	ctt	aat	gta	gca	768
Gly	Ala	Leu	Gly	Phe	Asp	Ser	Gln	Gly	Asn	Met	Gln	Leu	Asn	Val	Ala	
				245					250					255		
gga	gga	cta	agg	att	gat	tct	caa	aac	aga	cgc	ctt	ata	ctt	gat	gtt	816
Gly	Gly	Leu	Arg	Ile	Asp	Ser	Gln	Asn	Arg	Arg	Leu	Ile	Leu	Asp	Val	
		260						265					270			
agt	tat	ccg	ttt	gat	gct	caa	aac	caa	cta	aat	cta	aga	cta	gga	cag	864
Ser	Tyr	Pro	Phe	Asp	Ala	Gln	Asn	Gln	Leu	Asn	Leu	Arg	Leu	Gly	Gln	
		275					280					285				
ggc	cct	ctt	ttt	ata	aac	tca	gcc	cac	aac	ttg	gat	att	aac	tac	aac	912
Gly	Pro	Leu	Phe	Ile	Asn	Ser	Ala	His	Asn	Leu	Asp	Ile	Asn	Tyr	Asn	
	290					295					300					
aaa	ggc	ctt	tac	ttg	ttt	aca	gct	tca	aac	aat	tcc	aaa	aag	ctt	gag	960
Lys	Gly	Leu	Tyr	Leu	Phe	Thr	Ala	Ser	Asn	Asn	Ser	Lys	Lys	Leu	Glu	
305				310					315					320		
gtt	aac	cta	agc	act	gcc	aag	ggg	ttg	atg	ttt	gac	gct	aca	gcc	ata	1008
Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile	
				325				330						335		
gcc	att	aat	gca	gga	gat	ggg	ctt	gaa	ttt	ggg	tca	cct	aat	gca	cca	1056
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro	

-15-

			340				345				350						
aac Asn	aca Thr	aat Asn 355	ccc Pro	ctc Leu	aaa Lys	aca Thr	aaa Lys 360	att Ile	ggc Gly	cat His	ggc Gly	cta Leu 365	gaa Glu	ttt Phe	gat Asp	1104	
tca Ser	aac Asn 370	aag Lys	gct Ala	atg Met	gtt Val	cct Pro 375	aaa Lys	cta Leu	gga Gly	act Thr	ggc Gly 380	ctt Leu	agt Ser	ttt Phe	gac Asp	1152	
agc Ser 385	aca Thr	ggc Gly	gcc Ala	att Ile	aca Thr 390	gta Val	gga Gly	aac Asn	aaa Lys	aat Asn 395	aat Asn	gat Asp	aag Lys	cta Leu	act Thr 400	1200	
ttg Leu	tgg Trp	acc Thr	aca Thr 405	cca Pro	gct Ala	cca Pro	tct Ser	cct Pro	aac Asn 410	tgt Cys	tca Ser	cta Leu	aat Asn	gga Gly 415	ggc Gly	1248	
gga Gly	gat Asp	gct Ala	aaa Lys 420	ctc Leu	act Thr	ttg Leu	gtc Val	tta Leu 425	aca Thr	aaa Lys	tgt Cys	ggc Gly	agt Ser 430	caa Gln	ata Ile	1296	
ctt Leu	gct Ala	aca Thr 435	gtt Val	tca Ser	gtt Val	ttg Leu	gct Ala 440	gtt Val	aaa Lys	ggc Gly	agt Ser	ttg Leu 445	gct Ala	cca Pro	ata Ile	1344	
tct Ser	gga Gly 450	aca Thr	gtt Val	caa Gln	agt Ser	gct Ala 455	cat His	ctt Leu	att Ile	ata Ile	aga Arg 460	ttt Phe	gac Asp	gaa Glu	aat Asn	1392	
gga Gly 465	gtg Val	cta Leu	cta Leu	aac Asn	aat Asn 470	tcc Ser	ttc Phe	ctg Leu	gac Asp	cca Pro 475	gaa Glu	tat Tyr	tgg Trp	aac Asn	ttt Phe 480	1440	
aga Arg	aat Asn	gga Gly	gat Asp	ctt Leu 485	act Thr	gaa Glu	ggc Gly	aca Thr	gcc Ala 490	tat Tyr	aca Thr	aac Asn	gct Ala	gtt Val 495	gga Gly	1488	
ttt Phe	atg Met	cct Pro	aac Asn 500	cta Leu	tca Ser	gct Ala	tat Tyr	cca Pro 505	aaa Lys	tct Ser	cac His	ggt Gly	aaa Lys 510	act Thr	gcc Ala	1536	
aaa Lys	agt Ser	aac Asn 515	att Ile	gtc Val	agt Ser	caa Gln	gtt Val 520	tac Tyr	tta Leu	aac Asn	gga Gly	gac Asp 525	aaa Lys	act Thr	aaa Lys	1584	
cct Pro	gta Val 530	aca Thr	cta Leu	acc Thr	att Ile	aca Thr 535	cta Leu	aac Asn	ggt Gly	aca Thr	cag Gln 540	gaa Glu	aca Thr	gga Gly	gac Asp	1632	
aca Thr 545	act Thr	cca Pro	agt Ser	gca Ala	tac Tyr 550	tct Ser	atg Met	tca Ser	ttt Phe	tca Ser 555	tgg Trp	gac Asp	tgg Trp	tct Ser	ggc Gly 560	1680	
cac His	aac Asn	tac Tyr	att Ile	aat Asn 565	gaa Glu	ata Ile	ttt Phe	gcc Ala 570	aca Thr	tcc Ser	tct Ser	tac Tyr	act Thr	ttt Phe 575	tca Ser	1728	
tac Tyr	att Ile	gcc Ala	caa Gln 580	gaa Glu	taa *											1746	

-16-

<210> 8
 <211> 581
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5F KO12

<400> 8
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
 35 40 45
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
 85 90 95
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
 100 105 110
 Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
 115 120 125
 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
 130 135 140
 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
 145 150 155 160
 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
 165 170 175
 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
 180 185 190
 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
 195 200 205
 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
 210 215 220
 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
 225 230 235 240
 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
 245 250 255
 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
 260 265 270
 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
 275 280 285
 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
 290 295 300
 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
 305 310 315 320
 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
 325 330 335
 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
 340 345 350
 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
 355 360 365
 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
 370 375 380
 Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
 385 390 395 400

-17-

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Ser Leu Asn Gly Gly
 405 410 415
 Gly Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
 420 425 430
 Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
 435 440 445
 Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
 450 455 460
 Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
 465 470 475 480
 Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
 485 490 495
 Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
 500 505 510
 Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
 515 520 525
 Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
 530 535 540
 Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
 545 550 555 560
 His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
 565 570 575
 Tyr Ile Ala Gln Glu
 580

<210> 9
 <211> 1686
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5F S*

<221> CDS
 <222> (1)...(1746)

<400> 9
 acc ggt cct cca act gtg cct ttt ctt act cct ccc ttt gta tcc ccc 48
 Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro Phe Val Ser Pro
 1 5 10 15

 aat ggg ttt caa gag agt ccc cct ggg gta ctc tct ttg cgc cta tcc 96
 Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser Leu Arg Leu Ser
 20 25 30

 gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc aaa atg ggc aac 144
 Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu Lys Met Gly Asn
 35 40 45

 ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc caa aat gta acc 192
 Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser Gln Asn Val Thr
 50 55 60

 act gtg agc cca cct ctc gga gcc gga gcc tca aac ata aac ctg gaa 240
 Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn Ile Asn Leu Glu
 65 70 75 80

 ata tct gca ccc ctc aca gtt acc tca gaa gcc cta act gtg gct gcc 288
 Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu Thr Val Ala Ala
 85 90 95

-18-

gcc gca cct cta atg gtc gcg ggc aac aca ctc acc atg caa tca cag	336
Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr Met Gln Ser Gln	
100 105 110	
gcc ccg cta acc gtg cac gac tcc aaa ctt agc att gcc acc caa gga	384
Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile Ala Thr Gln Gly	
115 120 125	
ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa aca tca ggc ccc	432
Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln Thr Ser Gly Pro	
130 135 140	
ctc acc acc acc gat agc agt acc ctt act atc act gcc tca ccc cct	480
Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr Ala Ser Pro Pro	
145 150 155 160	
cta act act gcc act ggt agc ttg ggc att gac ttg aaa gag ccc att	528
Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu Lys Glu Pro Ile	
165 170 175	
tat aca caa aat gga aaa cta gga cta aag tac ggg gct cct ttg cat	576
Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly Ala Pro Leu His	
180 185 190	
gta aca gac gac cta aac act ttg acc gta gca act ggt cca ggt gtg	624
Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr Gly Pro Gly Val	
195 200 205	
act att aat aat act tcc ttg caa act aaa gtt act gga gcc ttg ggt	672
Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr Gly Ala Leu Gly	
210 215 220	
ttt gat tca caa ggc aat atg caa ctt aat gta gca gga gga cta agg	720
Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala Gly Gly Leu Arg	
225 230 235 240	
att gat tct caa aac aga cgc ctt ata ctt gat gtt agt tat ccg ttt	768
Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val Ser Tyr Pro Phe	
245 250 255	
gat gct caa aac caa cta aat cta aga cta gga cag ggc cct ctt ttt	816
Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln Gly Pro Leu Phe	
260 265 270	
ata aac tca gcc cac aac ttg gat att aac tac aac aaa ggc ctt tac	864
Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn Lys Gly Leu Tyr	
275 280 285	
ttg ttt aca gct tca aac aat tcc aaa aag ctt gag gtt aac cta agc	912
Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu Val Asn Leu Ser	
290 295 300	
act gcc aag ggg ttg atg ttt gac gct aca gcc ata gcc att aat gca	960
Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile Ala Ile Asn Ala	
305 310 315 320	
gga gat ggg ctt gaa ttt ggt tca cct aat gca cca aac aca aat ccc	1008
Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro Asn Thr Asn Pro	
325 330 335	

c	t	a	a	a	a	a	c	g	c	a	t	t	a	a	a		1056
Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp	Ser	Asn	Lys	Ala		
			340					345					350				
a	t	c	a	a	c	a	c	t	a	t	t	a	a	a	c		1104
Met	Val	Pro	Lys	Leu	Gly	Thr	Gly	Leu	Ser	Phe	Asp	Ser	Thr	Gly	Ala		
		355					360					365					
a	a	t	a	a	a	a	a	a	a	c	a	a	t	t	a		1152
Ile	Thr	Val	Gly	Asn	Lys	Asn	Asn	Asp	Lys	Leu	Thr	Leu	Trp	Thr	Thr		
	370					375					380						
c	c	a	t	c	a	t	a	c	a	a	g	a	a	a	a		1200
Pro	Ala	Pro	Ser	Pro	Asn	Cys	Arg	Leu	Asn	Ala	Glu	Lys	Asp	Ala	Lys		
385					390					395					400		
c	t	a	t	t	a	a	t	g	a	a	c	a	t	a	c		1248
Leu	Thr	Leu	Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile	Leu	Ala	Thr	Val		
				405					410					415			
t	c	a	t	t	a	a	g	t	t	g	c	a	t	t	g		1296
Ser	Val	Leu	Ala	Val	Lys	Gly	Ser	Leu	Ala	Pro	Ile	Ser	Gly	Thr	Val		
			420					425					430				
c	a	a	c	c	a	t	a	a	g	a	a	a	g	t	c		1344
Gln	Ser	Ala	His	Leu	Ile	Ile	Arg	Phe	Asp	Glu	Asn	Gly	Val	Leu	Leu		
		435					440					445					
a	a	c	t	c	g	c	c	a	t	t	a	t	a	g	a		1392
Asn	Asn	Ser	Phe	Leu	Asp	Pro	Glu	Tyr	Trp	Asn	Phe	Arg	Asn	Gly	Asp		
	450					455					460						
c	a	c	g	a	c	t	a	a	c	g	t	g	t	a	c		1440
Leu	Thr	Glu	Gly	Thr	Ala	Tyr	Thr	Asn	Ala	Val	Gly	Phe	Met	Pro	Asn		
	465				470					475					480		
c	t	a	t	c	a	t	c	g	a	a	c	g	a	a	a		1488
Leu	Ser	Ala	Tyr	Pro	Lys	Ser	His	Gly	Lys	Thr	Ala	Lys	Ser	Asn	Ile		
				485					490					495			
g	t	c	a	t	t	a	a	g	a	a	a	c	c	t	a		1536
Val	Ser	Gln	Val	Tyr	Leu	Asn	Gly	Asp	Lys	Thr	Lys	Pro	Val	Thr	Leu		
			500					505					510				
a	c	a	c	a	g	a	c	a	a	g	a	a	a	c	c		1584
Thr	Ile	Thr	Leu	Asn	Gly	Thr	Gln	Glu	Thr	Gly	Asp	Thr	Thr	Pro	Ser		
		515					520					525					
g	a	t	a	t	t	t	c	t	g	t	g	c	c	a	a		1632
Ala	Tyr	Ser	Met	Ser	Phe	Ser	Trp	Asp	Trp	Ser	Gly	His	Asn	Tyr	Ile		
	530					535					540						
a	a	a	t	g	a	c	t	t	a	t	t	c	t	g	c		1680
Asn	Glu	Ile	Phe	Ala	Thr	Ser	Ser	Tyr	Thr	Phe	Ser	Tyr	Ile				

-20-

* * * * *

<210> 10
 <211> 561
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5F S*

<400> 10
 Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro Phe Val Ser Pro
 1 5 10 15
 Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser Leu Arg Leu Ser
 20 25 30
 Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu Lys Met Gly Asn
 35 40 45
 Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser Gln Asn Val Thr
 50 55 60
 Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn Ile Asn Leu Glu
 65 70 75 80
 Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu Thr Val Ala Ala
 85 90 95
 Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr Met Gln Ser Gln
 100 105 110
 Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile Ala Thr Gln Gly
 115 120 125
 Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln Thr Ser Gly Pro
 130 135 140
 Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr Ala Ser Pro Pro
 145 150 155 160
 Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu Lys Glu Pro Ile
 165 170 175
 Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly Ala Pro Leu His
 180 185 190
 Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr Gly Pro Gly Val
 195 200 205
 Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr Gly Ala Leu Gly
 210 215 220
 Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala Gly Gly Leu Arg
 225 230 235 240
 Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val Ser Tyr Pro Phe
 245 250 255
 Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln Gly Pro Leu Phe
 260 265 270
 Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn Lys Gly Leu Tyr
 275 280 285
 Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu Val Asn Leu Ser
 290 295 300
 Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile Ala Ile Asn Ala
 305 310 315 320
 Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro Asn Thr Asn Pro
 325 330 335
 Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp Ser Asn Lys Ala
 340 345 350
 Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp Ser Thr Gly Ala
 355 360 365
 Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr Leu Trp Thr Thr
 370 375 380

-21-

```

Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu Lys Asp Ala Lys
385          390          395          400
Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Thr Val
          405          410          415
Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr Val
          420          425          430
Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn Gly Val Leu Leu
          435          440          445
Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe Arg Asn Gly Asp
          450          455          460
Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro Asn
465          470          475          480
Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala Lys Ser Asn Ile
          485          490          495
Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Val Thr Leu
          500          505          510
Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp Thr Thr Pro Ser
          515          520          525
Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile
          530          535          540
Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln
545          550          555          560
Glu

```

<210> 11
 <211> 1776
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5F S*RGD

<221> CDS
 <222> (1)...(1746)

```

<400> 11
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1          5          10          15

tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
          20          25          30

ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
          35          40          45

ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
          50          55          60

aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
          65          70          75          80

caa aat gta acc act gtg agc cca cct ctc gga gcc gga gcc tca aac 288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn
          85          90          95

```

-22-

ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta	336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu	
100 105 110	
act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc	384
Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr	
115 120 125	
atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att	432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile	
130 135 140	
gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa	480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln	
145 150 155 160	
aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act	528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr	
165 170 175	
gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg	576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu	
180 185 190	
aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg	624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly	
195 200 205	
gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act	672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr	
210 215 220	
ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act	720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr	
225 230 235 240	
gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca	768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala	
245 250 255	
gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt	816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val	
260 265 270	
agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag	864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln	
275 280 285	
ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac	912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn	
290 295 300	
aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag	960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu	
305 310 315 320	
gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata	1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile	
325 330 335	

-23-

gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca	1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro	
340 345 350	
aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat	1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp	
355 360 365	
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac	1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp	
370 375 380	
agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act	1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr	
385 390 395 400	
ttg tgg acc aca cca gct cca tct cct aac tgt aga cta aat gca gag	1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu	
405 410 415	
aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata	1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile	
420 425 430	
ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata	1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile	
435 440 445	
tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat	1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn	
450 455 460	
gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt	1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe	
465 470 475 480	
aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga	1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly	
485 490 495	
ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc	1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala	
500 505 510	
aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa	1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys	
515 520 525	
cct gta aca cta acc att aca cta aac ggt aca cag gaa aca ggt gat	1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp	
530 535 540	
cat tgt gat tgt cgt ggt gat tgt ttt tgt aca act cca agt gca tac	1680
His Cys Asp Cys Arg Gly Asp Cys Phe Cys Thr Thr Pro Ser Ala Tyr	
545 550 555 560	
tct atg tca ttt tca tgg gac tgg tct ggc cac aac tac att aat gaa	1728
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu	
565 570 575	
ata ttt gcc aca tcc tct tacacttttt catacattgc ccaagaataa	1776

-24-

Ile Phe Ala Thr Ser Ser
580

<210> 12
<211> 582
<212> PRT
<213> Artificial Sequence

<220>
<223> 5F S*RGD

<400> 12
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn
85 90 95
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100 105 110
Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115 120 125
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130 135 140
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145 150 155 160
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165 170 175
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180 185 190
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195 200 205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210 215 220
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225 230 235 240
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp

-25-

370		375		380
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr				
385		390		395
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu				
	405		410	
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile				
	420		425	
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile				
	435		440	
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn				
	450		455	
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe				
465		470		475
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly				
	485		490	
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala				
	500		505	
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys				
	515		520	
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp				
	530		535	
His Cys Asp Cys Arg Gly Asp Cys Phe Cys Thr Thr Pro Ser Ala Tyr				
545		550		555
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu				
	565		570	
Ile Phe Ala Thr Ser Ser				
	580			

<210> 13
 <211> 1746
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5F KO1S*

<221> CDS
 <222> (1)...(1746)

<400> 13	
atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca	48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro	
1 5 10 15	
tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc	96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro	
20 25 30	
ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct	144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser	
35 40 45	
ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc	192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu	
50 55 60	
aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc	240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser	
65 70 75 80	

-26-

caa aat gta acc act gtg agc cca cct ctc gga gcc gga gcc tca aac	288
Gln Asn Val Thr Thr 85 Val Ser Pro Pro Leu 90 Gly Ala Gly Ala Ser 95 Asn	
ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta	336
Ile Asn Leu 100 Glu Ile Ser Ala Pro Leu 105 Thr Val Thr Ser Glu Ala Leu 110	
act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc	384
Thr Val 115 Ala Ala Ala Ala Pro Leu 120 Met Val Ala Gly Asn 125 Thr Leu Thr	
atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att	432
Met Gln Ser Gln Ala Pro Leu 135 Thr Val His Asp Ser 140 Lys Leu Ser Ile	
gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa	480
Ala Thr Gln Gly Pro Leu 150 Thr Val Ser Glu Gly Lys Leu Ala Leu Gln 160	
aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act	528
Thr Ser Gly Pro 165 Thr Thr Thr Asp 170 Ser Ser Thr Leu Thr 175 Ile Thr	
gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg	576
Ala Ser Pro 180 Leu Thr Thr Ala Thr 185 Gly Ser Leu Gly Ile 190 Asp Leu	
aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg	624
Lys Glu Pro 195 Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly 205	
gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act	672
Ala Pro Leu His Val Thr 215 Asp Asp Leu Asn Thr 220 Leu Thr Val Ala Thr	
ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act	720
Gly Pro Gly Val Thr 230 Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr 240	
gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca	768
Gly Ala Leu Gly Phe 245 Asp Ser Gln Gly Asn 250 Met Gln Leu Asn 255 Val Ala	
gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt	816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val 270	
agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag	864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu 285 Arg Leu Gly Gln	
ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac	912
Gly Pro Leu Phe Ile Asn 295 Ser Ala His Asn Leu 300 Asp Ile Asn Tyr Asn	
aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag	960
Lys Gly Leu Tyr Leu 310 Phe Thr Ala Ser Asn Asn 315 Ser Lys Lys Leu Glu 320	
ggt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata	1008

-27-

Val	Asn	Leu	Ser	Thr	Ala	Lys	Gly	Leu	Met	Phe	Asp	Ala	Thr	Ala	Ile	
				325					330					335		
gcc	att	aat	gca	gga	gat	ggg	ctt	gaa	ttt	ggc	tca	cct	aat	gca	cca	1056
Ala	Ile	Asn	Ala	Gly	Asp	Gly	Leu	Glu	Phe	Gly	Ser	Pro	Asn	Ala	Pro	
			340					345					350			
aac	aca	aat	ccc	ctc	aaa	aca	aaa	att	ggc	cat	ggc	cta	gaa	ttt	gat	1104
Asn	Thr	Asn	Pro	Leu	Lys	Thr	Lys	Ile	Gly	His	Gly	Leu	Glu	Phe	Asp	
		355					360					365				
tca	aac	aag	gct	atg	gtt	cct	aaa	cta	gga	act	ggc	ctt	agt	ttt	gac	1152
Ser	Asn	Lys	Ala	Met	Val	Pro	Lys	Leu	Gly	Thr	Gly	Leu	Ser	Phe	Asp	
	370					375					380					
agc	aca	ggc	gcc	att	aca	gta	gga	aac	aaa	aat	aat	gat	aag	cta	act	1200
Ser	Thr	Gly	Ala	Ile	Thr	Val	Gly	Asn	Lys	Asn	Asn	Asp	Lys	Leu	Thr	
385					390					395					400	
ttg	tgg	acc	aca	cca	gct	cca	gag	gct	aac	tgt	aga	cta	aat	gca	gag	1248
Leu	Trp	Thr	Thr	Pro	Ala	Pro	Glu	Ala	Asn	Cys	Arg	Leu	Asn	Ala	Glu	
				405					410					415		
aaa	gat	gct	aaa	ctc	act	ttg	gtc	tta	aca	aaa	tgt	ggc	agt	caa	ata	1296
Lys	Asp	Ala	Lys	Leu	Thr	Leu	Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile	
			420					425					430			
ctt	gct	aca	gtt	tca	gtt	ttg	gct	gtt	aaa	ggc	agt	ttg	gct	cca	ata	1344
Leu	Ala	Thr	Val	Ser	Val	Leu	Ala	Val	Lys	Gly	Ser	Leu	Ala	Pro	Ile	
		435				440						445				
tct	gga	aca	gtt	caa	agt	gct	cat	ctt	att	ata	aga	ttt	gac	gaa	aat	1392
Ser	Gly	Thr	Val	Gln	Ser	Ala	His	Leu	Ile	Ile	Arg	Phe	Asp	Glu	Asn	
	450					455					460					
gga	gtg	cta	cta	aac	aat	tcc	ttc	ctg	gac	cca	gaa	tat	tgg	aac	ttt	1440
Gly	Val	Leu	Leu	Asn	Asn	Ser	Phe	Leu	Asp	Pro	Glu	Tyr	Trp	Asn	Phe	
465				470						475					480	
aga	aat	gga	gat	ctt	act	gaa	ggc	aca	gcc	tat	aca	aac	gct	gtt	gga	1488
Arg	Asn	Gly	Asp	Leu	Thr	Glu	Gly	Thr	Ala	Tyr	Thr	Asn	Ala	Val	Gly	
				485				490						495		
ttt	atg	cct	aac	cta	tca	gct	tat	cca	aaa	tct	cac	ggc	aaa	act	gcc	1536
Phe	Met	Pro	Asn	Leu	Ser	Ala	Tyr	Pro	Lys	Ser	His	Gly	Lys	Thr	Ala	
			500					505					510			
aaa	agt	aac	att	gtc	agt	caa	gtt	tac	tta	aac	gga	gac	aaa	act	aaa	1584
Lys	Ser	Asn	Ile	Val	Ser	Gln	Val	Tyr	Leu	Asn	Gly	Asp	Lys	Thr	Lys	
		515					520					525				
cct	gta	aca	cta	acc	att	aca	cta	aac	ggc	aca	cag	gaa	aca	gga	gac	1632
Pro	Val	Thr	Leu	Thr	Ile	Thr	Leu	Asn	Gly	Thr	Gln	Glu	Thr	Gly	Asp	
	530					535					540					
aca	act	cca	agt	gca	tac	tct	atg	tca	ttt	tca	tgg	gac	tgg	tct	ggc	1680
Thr	Thr	Pro	Ser	Ala	Tyr	Ser	Met	Ser	Phe	Ser	Trp	Asp	Trp	Ser	Gly	
545					550					555					560	
cac	aac	tac	att	aat	gaa	ata	ttt	gcc	aca	tcc	tct	tac	act	ttt	tca	1728
His	Asn	Tyr	Ile	Asn	Glu	Ile	Phe	Ala	Thr	Ser	Ser	Tyr	Thr	Phe	Ser	

-28-

565
 tac att gcc caa gaa taa
 Tyr Ile Ala Gln Glu *
 580

570

575

1746

<210> 14
 <211> 581
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5F KO1S*

<400> 14
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
 35 40 45
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn
 85 90 95
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
 100 105 110
 Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
 115 120 125
 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
 130 135 140
 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
 145 150 155 160
 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
 165 170 175
 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
 180 185 190
 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
 195 200 205
 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
 210 215 220
 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
 225 230 235 240
 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
 245 250 255
 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
 260 265 270
 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
 275 280 285
 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
 290 295 300
 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
 305 310 315 320
 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
 325 330 335
 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
 340 345 350
 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

-29-

[illegible]

```
<210> 15
<211> 1776
<212> DNA
<213> Artificial Sequence
```

<220>
<223> 5F KO1S*RGD

```
<221> CDS
<222> (1) ... (1776)
```

<400> 15																	
atg	aag	cgc	gca	aga	ccg	tct	gaa	gat	acc	ttc	aac	ccc	gtg	tat	cca	48	
Met	Lys	Arg	Ala	Arg	Pro	Ser	Glu	Asp	Thr	Phe	Asn	Pro	Val	Tyr	Pro		
1				5					10					15			
tat	gac	acg	gaa	acc	ggg	cct	cca	act	gtg	cct	ttt	ctt	act	cct	ccc	96	
Tyr	Asp	Thr	Glu	Thr	Gly	Pro	Pro	Thr	Val	Pro	Phe	Leu	Thr	Pro	Pro		
			20					25					30				
ttt	gta	tcc	ccc	aat	ggg	ttt	caa	gag	agt	ccc	cct	ggg	gta	ctc	tct	144	
Phe	Val	Ser	Pro	Asn	Gly	Phe	Gln	Glu	Ser	Pro	Pro	Gly	Val	Leu	Ser		
		35					40					45					
ttg	cgc	cta	tcc	gaa	cct	cta	gtt	acc	tcc	aat	ggc	atg	ctt	gcg	ctc	192	
Leu	Arg	Leu	Ser	Glu	Pro	Leu	Val	Thr	Ser	Asn	Gly	Met	Leu	Ala	Leu		
	50					55					60						
aaa	atg	ggc	aac	ggc	ctc	tct	ctg	gac	gag	gcc	ggc	aac	ctt	acc	tcc	240	
Lys	Met	Gly	Asn	Gly	Leu	Ser	Leu	Asp	Glu	Ala	Gly	Asn	Leu	Thr	Ser		

-30-

65					70					75					80					
caa Gln	aat Asn	gta Val	acc Thr	act Thr 85	gtg Val	agc Ser	cca Pro	cct Pro	ctc Leu 90	gga Gly	gcc Ala	gga Gly	gcc Ala	tca Ser 95	aac Asn	288				
ata Ile	aac Asn	ctg Leu	gaa Glu 100	ata Ile	tct Ser	gca Ala	ccc Pro	ctc Leu 105	aca Thr	gtt Val	acc Thr	tca Ser	gaa Glu 110	gcc Ala	cta Leu	336				
act Thr	gtg Val	gct Ala 115	gcc Ala	gcc Ala	gca Ala	cct Pro	cta Leu 120	atg Met	gtc Val	gcg Ala	ggc Gly	aac Asn 125	aca Thr	ctc Leu	acc Thr	384				
atg Met	caa Gln 130	tca Ser	cag Gln	gcc Ala	ccg Pro	cta Leu 135	acc Thr	gtg Val	cac His	gac Asp	tcc Ser 140	aaa Lys	ctt Leu	agc Ser	att Ile	432				
gcc Ala 145	acc Thr	caa Gln	gga Gly	ccc Pro	ctc Leu 150	aca Thr	gtg Val	tca Ser	gaa Glu	gga Gly 155	aag Lys	cta Leu	gcc Ala	ctg Leu	caa Gln 160	480				
aca Thr	tca Ser	ggc Gly	ccc Pro	ctc Leu 165	acc Thr	acc Thr	acc Thr	gat Asp	agc Ser 170	agt Ser	acc Thr	ctt Leu	act Thr	atc Ile 175	act Thr	528				
gcc Ala	tca Ser	ccc Pro	cct Pro 180	cta Leu	act Thr	act Thr	gcc Ala	act Thr 185	ggg Gly	agc Ser	ttg Leu	ggc Gly	att Ile 190	gac Asp	ttg Leu	576				
aaa Lys	gag Glu	ccc Pro 195	att Ile	tat Tyr	aca Thr	caa Gln	aat Asn 200	gga Gly	aaa Lys	cta Leu	gga Gly	cta Leu 205	aag Lys	tac Tyr	ggg Gly	624				
gct Ala	cct Pro 210	ttg Leu	cat His	gta Val	aca Thr	gac Asp 215	gac Asp	cta Leu	aac Asn	act Thr	ttg Leu 220	acc Thr	gta Val	gca Ala	act Thr	672				
ggg Gly 225	cca Pro	ggg Gly	gtg Val	act Thr	att Ile 230	aat Asn	aat Asn	act Thr	tcc Ser	ttg Leu 235	caa Gln	act Thr	aaa Lys	gtt Val	act Thr 240	720				
gga Gly	gcc Ala	ttg Leu	ggg Gly	ttt Phe 245	gat Asp	tca Ser	caa Gln	ggc Gly	aat Asn 250	atg Met	caa Gln	ctt Leu	aat Asn	gta Val 255	gca Ala	768				
gga Gly	gga Gly	cta Leu	agg Arg 260	att Ile	gat Asp	tct Ser	caa Gln	aac Asn 265	aga Arg	cgc Arg	ctt Leu	ata Ile	ctt Leu 270	gat Asp	gtt Val	816				
agt Ser	tat Tyr	ccg Pro 275	ttt Phe	gat Asp	gct Ala	caa Gln	aac Asn 280	caa Gln	cta Leu	aat Asn	cta Leu	aga Arg 285	cta Leu	gga Gly	cag Gln	864				
ggc Gly	cct Pro 290	ctt Leu	ttt Phe	ata Ile	aac Asn	tca Ser 295	gcc Ala	cac His	aac Asn	ttg Leu 300	gat Asp	att Ile	aac Asn	tac Tyr	aac Asn	912				
aaa Lys 305	ggc Gly	ctt Leu	tac Tyr	ttg Leu	ttt Phe 310	aca Thr	gct Ala	tca Ser	aac Asn	aat Asn 315	tcc Ser	aaa Lys	aag Lys	ctt Leu	gag Glu 320	960				

-31-

gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata	1008
Val Asn Leu Ser Thr 325 Ala Lys Gly Leu Met 330 Phe Asp Ala Thr 335 Ile	
gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca	1056
Ala Ile Asn Ala 340 Gly Asp Gly Leu Glu 345 Phe Gly Ser Pro 350 Asn Ala Pro	
aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat	1104
Asn Thr 355 Asn Pro Leu Lys Thr 360 Lys Ile Gly His Gly Leu 365 Glu Phe Asp	
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac	1152
Ser Asn Lys Ala Met Val 375 Pro Lys Leu Gly Thr 380 Gly Leu Ser Phe Asp	
agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act	1200
Ser Thr Gly Ala Ile Thr 390 Val Gly Asn Lys Asn 395 Asn Asp Lys Leu Thr 400	
ttg tgg acc aca cca gct cca gag gct aac tgt aga cta aat gca gag	1248
Leu Trp Thr Thr 405 Pro Ala Pro Glu Ala Asn 410 Cys Arg Leu Asn 415 Ala Glu	
aaa gat gct aaa ctc act ttg gtc tta aca aaa tgt ggc agt caa ata	1296
Lys Asp Ala Lys 420 Leu Thr Leu Val 425 Thr Lys Cys Gly Ser 430 Gln Ile	
ctt gct aca gtt tca gtt ttg gct gtt aaa ggc agt ttg gct cca ata	1344
Leu Ala Thr 435 Val Ser Val Leu Ala Val 440 Lys Gly Ser Leu 445 Ala Pro Ile	
tct gga aca gtt caa agt gct cat ctt att ata aga ttt gac gaa aat	1392
Ser Gly Thr Val Gln Ser 455 Ala His Leu Ile Ile 460 Arg Phe Asp Glu Asn	
gga gtg cta cta aac aat tcc ttc ctg gac cca gaa tat tgg aac ttt	1440
Gly Val Leu Leu Asn 470 Ser Phe Leu Asp 475 Pro Glu Tyr Trp Asn 480 Phe	
aga aat gga gat ctt act gaa ggc aca gcc tat aca aac gct gtt gga	1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr 490 Ala Tyr Thr Asn Ala 495 Val Gly	
ttt atg cct aac cta tca gct tat cca aaa tct cac ggt aaa act gcc	1536
Phe Met Pro Asn Leu Ser Ala Tyr 505 Pro Lys Ser His Gly Lys 510 Thr Ala	
aaa agt aac att gtc agt caa gtt tac tta aac gga gac aaa act aaa	1584
Lys Ser Asn Ile Val Ser Gln 520 Val Tyr Leu Asn Gly 525 Asp Lys Thr Lys	
cct gta aca cta acc att aca cta aac ggt aca cag gaa aca ggt gat	1632
Pro Val Thr Leu Thr Ile Thr 535 Leu Asn Gly Thr 540 Gln Glu Thr Gly Asp	
cat tgt gat tgt cgt ggt gat tgt ttt tgt aca act cca agt gca tac	1680
His Cys Asp Cys Arg Gly 550 Asp Cys Phe Cys Thr 555 Thr Pro Ser Ala Tyr 560	

-32-

```

tct atg tca ttt tca tgg gac tgg tct ggc cac aac tac att aat gaa 1728
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu
                    565                    570                    575

ata ttt gcc aca tcc tct tac act ttt tca tac att gcc caa gaa taa 1776
Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu *
                    580                    585                    590

```

```

<210> 16
<211> 591
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> 5F K01S*RGD

```

```

<400> 16
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1      5      10
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
      20      25      30
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
      35      40      45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
      50      55      60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
      65      70      75      80
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Gly Ala Gly Ala Ser Asn
      85      90      95
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
      100      105      110
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
      115      120      125
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
      130      135      140
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
      145      150      155      160
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
      165      170      175
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
      180      185      190
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
      195      200      205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
      210      215      220
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
      225      230      235      240
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
      245      250      255
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
      260      265      270
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
      275      280      285
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
      290      295      300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
      305      310      315      320
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
      325      330      335

```

-33-

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
 340 345 350
 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
 355 360 365
 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
 370 375 380
 Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
 385 390 395 400
 Leu Trp Thr Thr Pro Ala Pro Glu Ala Asn Cys Arg Leu Asn Ala Glu
 405 410 415
 Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
 420 425 430
 Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
 435 440 445
 Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
 450 455 460
 Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
 465 470 475 480
 Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
 485 490 495
 Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
 500 505 510
 Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
 515 520 525
 Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
 530 535 540
 His Cys Asp Cys Arg Gly Asp Cys Phe Cys Thr Thr Pro Ser Ala Tyr
 545 550 555 560
 Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu
 565 570 575
 Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu
 580 585 590

<210> 17
 <211> 972
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad35 fiber

<221> CDS
 <222> (1)...(972)

<400> 17
 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta 144
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa 192
 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60

-34-

gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa	240
Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu	
65 70 75 80	
aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa	288
Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu	
85 90 95	
cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc	336
Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala	
100 105 110	
aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag	384
Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys	
115 120 125	
gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt	432
Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys	
130 135 140	
caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta	480
Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val	
145 150 155 160	
tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt	528
Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly	
165 170 175	
gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc	576
Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile	
180 185 190	
caa tta aga tta tat ttt gac tct tct gga aat cta tta act gag gaa	624
Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu	
195 200 205	
tca gac tta aaa att cca ctt aaa aat aaa tct tct aca gcg acc agt	672
Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser	
210 215 220	
gaa act gta gcc agc agc aaa gcc ttt atg cca agt act aca gct tat	720
Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr	
225 230 235 240	
ccc ttc aac acc act act agg gat agt gaa aac tac att cat gga ata	768
Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile	
245 250 255	
tgt tac tac atg act agt tat gat aga agt cta ttt ccc ttg aac att	816
Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile	
260 265 270	
tct ata atg cta aac agc cgt atg att tct tcc aat gtt gcc tat gcc	864
Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala	
275 280 285	
ata caa ttt gaa tgg aat cta aat gca agt gaa tct cca gaa agc aac	912
Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn	
290 295 300	

-35-

ata gct acg ctg acc aca tcc ccc ttt ttc ttt tct tac att aca gaa 960
 ile ala thr leu thr thr ser pro phe phe phe ser tyr ile thr glu
 305 310 315 320
 gac gac gaa taa 972
 asp asp glu *

<210> 18
 <211> 323
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ad 35 fiber

<400> 18
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90 95
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125
 Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
 130 135 140
 Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
 145 150 155 160
 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly
 165 170 175
 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
 180 185 190
 Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu
 195 200 205
 Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser
 210 215 220
 Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr
 225 230 235 240
 Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile
 245 250 255
 Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile
 260 265 270
 Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala
 275 280 285
 Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn
 290 295 300
 Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu
 305 310 315 320
 Asp Asp Glu

-36-

<210> 19
 <211> 1002
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 35F RGD

<221> CDS
 <222> (1)...(1002)

<400> 19
 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15

tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30

att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta 144
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45

aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa 192
 Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60

gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa 240
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80

aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa 288
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90 95

cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc 336
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110

aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag 384
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125

gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt 432
 Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
 130 135 140

caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta 480
 Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
 145 150 155 160

tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt 528
 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly
 165 170 175

gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc 576
 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
 180 185 190

caa tta aga tta tat ttt gac tct tct gga aat cta tta act gag gaa 624

-37-

Gln	Leu	Arg	Leu	Tyr	Phe	Asp	Ser	Ser	Gly	Asn	Leu	Leu	Thr	Glu	Glu		
		195					200					205					
tca	gac	tta	aaa	att	cca	ctt	aaa	aat	aaa	tct	tct	aca	gcg	acc	agt	672	
Ser	Asp	Leu	Lys	Ile	Pro	Leu	Lys	Asn	Lys	Ser	Ser	Thr	Ala	Thr	Ser		
		210				215					220						
gaa	act	gta	gcc	agc	agc	aaa	gcc	ttt	atg	cca	agt	act	aca	gct	tat	720	
Glu	Thr	Val	Ala	Ser	Ser	Lys	Ala	Phe	Met	Pro	Ser	Thr	Thr	Ala	Tyr		
		225				230				235					240		
ccc	ttc	aac	acc	act	act	agg	gat	agt	gaa	aac	tac	att	cat	gga	ata	768	
Pro	Phe	Asn	Thr	Thr	Thr	Arg	Asp	Ser	Glu	Asn	Tyr	Ile	His	Gly	Ile		
				245					250					255			
tgt	tac	tac	atg	act	agt	tat	gat	aga	agt	cta	ttt	ccc	ttg	aac	att	816	
Cys	Tyr	Tyr	Met	Thr	Ser	Tyr	Asp	Arg	Ser	Leu	Phe	Pro	Leu	Asn	Ile		
			260					265					270				
tct	ata	atg	cta	aac	agc	cgt	atg	att	tct	tcc	aat	gta	cat	tgt	gat	864	
Ser	Ile	Met	Leu	Asn	Ser	Arg	Met	Ile	Ser	Ser	Asn	Val	His	Cys	Asp		
		275					280					285					
tgt	cgt	ggc	gat	tgt	ttt	tgc	gca	tat	gcc	ata	caa	ttt	gaa	tggt	aat	912	
Cys	Arg	Gly	Asp	Cys	Phe	Cys	Ala	Tyr	Ala	Ile	Gln	Phe	Glu	Trp	Asn		
		290				295					300						
cta	aat	gca	agt	gaa	tct	cca	gaa	agc	aac	ata	gct	acg	ctg	acc	aca	960	
Leu	Asn	Ala	Ser	Glu	Ser	Pro	Glu	Ser	Asn	Ile	Ala	Thr	Leu	Thr	Thr		
		305				310				315					320		
tcc	ccc	ttt	ttc	ttt	tct	tac	att	aca	gaa	gac	gac	gaa	taa			1002	
Ser	Pro	Phe	Phe	Phe	Ser	Tyr	Ile	Thr	Glu	Asp	Asp	Glu	*				
				325					330								

<210> 20
 <211> 332
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 35FRGD

<400> 20
 Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro Tyr
 1 5 10 15
 Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe Ile
 20 25 30
 Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu Lys
 35 40 45
 Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys Val
 50 55 60
 Gly Gly Gly Leu Thr Val Asp Thr Asp Gly Thr Leu Gln Glu Asn
 65 70 75 80
 Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu Leu
 85 90 95
 Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala Lys
 100 105 110
 Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys Asp

-38-

```

      115      120      125
Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Asn Cys Gln
      130      135      140
Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val Leu
145      150      155      160
Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly Val
      165      170      175
Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile Gln
      180      185      190
Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu Ser
      195      200      205
Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser Glu
      210      215      220
Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr Pro
225      230      235      240
Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile Cys
      245      250      255
Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile Ser
      260      265      270
Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val His Cys Asp Cys
      275      280      285
Arg Gly Asp Cys Phe Cys Ala Tyr Ala Ile Gln Phe Glu Trp Asn Leu
      290      295      300
Asn Ala Ser Glu Ser Pro Glu Ser Asn Ile Ala Thr Leu Thr Thr Ser
305      310      315      320
Pro Phe Phe Phe Ser Tyr Ile Thr Glu Asp Asp Glu
      325      330

```

<210> 21
 <211> 1164
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad41 short fiber

<221> CDS
 <222> (1)...(1164)

```

<400> 21
atg aaa aga acc aga att gaa gac gac ttc aac ccc gtc tac ccc tat   48
Met Lys Arg Thr Arg Ile Glu Asp Asp Phe Asn Pro Val Tyr Pro Tyr
  1          5          10          15

gac acc ttc tca act ccc agc atc ccc tat gta gct ccg ccc ttc gtt   96
Asp Thr Phe Ser Thr Pro Ser Ile Pro Tyr Val Ala Pro Pro Phe Val
          20          25          30

tct tct gac ggg tta cag gaa aaa ccc cca gga gtt tta gca ctc aag   144
Ser Ser Asp Gly Leu Gln Glu Lys Pro Pro Gly Val Leu Ala Leu Lys
          35          40          45

tac act gac ccc att act acc aat gct aag cat gag ctt act tta aaa   192
Tyr Thr Asp Pro Ile Thr Thr Asn Ala Lys His Glu Leu Thr Leu Lys
          50          55          60

ctt gga agc aac ata act tta gaa aat ggg tta ctt tcg gcc aca gtt   240
Leu Gly Ser Asn Ile Thr Leu Glu Asn Gly Leu Leu Ser Ala Thr Val
  65          70          75          80

```

-39-

ccc	act	gtt	tct	cct	ccc	ctt	aca	aac	agt	aac	aac	tcc	ctg	ggg	tta	288
Pro	Thr	Val	Ser	Pro	Pro	Leu	Thr	Asn	Ser	Asn	Asn	Ser	Leu	Gly	Leu	
				85					90					95		
gcc	aca	tcc	gct	ccc	ata	gct	gta	tca	gct	aac	tct	ctc	aca	ttg	gcc	336
Ala	Thr	Ser	Ala	Pro	Ile	Ala	Val	Ser	Ala	Asn	Ser	Leu	Thr	Leu	Ala	
			100					105					110			
acc	gcc	gca	cca	ctg	aca	gta	agc	aac	aac	cag	ctt	agt	att	aac	gcg	384
Thr	Ala	Ala	Pro	Leu	Thr	Val	Ser	Asn	Asn	Gln	Leu	Ser	Ile	Asn	Ala	
		115					120					125				
ggc	aga	ggg	tta	gtt	ata	act	aac	aat	gcc	tta	aca	gtt	aat	cct	acc	432
Gly	Arg	Gly	Leu	Val	Ile	Thr	Asn	Asn	Ala	Leu	Thr	Val	Asn	Pro	Thr	
	130					135						140				
gga	gcg	cta	ggg	ttc	aat	aac	aca	gga	gct	tta	caa	tta	aat	gct	gca	480
Gly	Ala	Leu	Gly	Phe	Asn	Asn	Thr	Gly	Ala	Leu	Gln	Leu	Asn	Ala	Ala	
145					150					155					160	
gga	gga	atg	aga	gtg	gac	ggg	gcc	aac	tta	att	ctt	cat	gta	gca	tat	528
Gly	Gly	Met	Arg	Val	Asp	Gly	Ala	Asn	Leu	Ile	Leu	His	Val	Ala	Tyr	
				165					170					175		
ccc	ttt	gaa	gca	atc	aac	cag	cta	aca	ctg	cga	tta	gaa	aac	ggg	tta	576
Pro	Phe	Glu	Ala	Ile	Asn	Gln	Leu	Thr	Leu	Arg	Leu	Glu	Asn	Gly	Leu	
			180					185					190			
gaa	gta	acc	agc	gga	gga	aag	ctt	aac	gtt	aag	ttg	gga	tca	ggc	ctc	624
Glu	Val	Thr	Ser	Gly	Gly	Lys	Leu	Asn	Val	Lys	Leu	Gly	Ser	Gly	Leu	
		195					200					205				
caa	ttt	gac	agt	aac	gga	cgc	att	gct	att	agt	aat	agc	aac	cga	act	672
Gln	Phe	Asp	Ser	Asn	Gly	Arg	Ile	Ala	Ile	Ser	Asn	Ser	Asn	Arg	Thr	
	210					215					220					
cga	agt	gta	cca	tcc	ctc	act	acc	att	tggt	tct	atc	tcg	cct	acg	cct	720
Arg	Ser	Val	Pro	Ser	Leu	Thr	Thr	Ile	Trp	Ser	Ile	Ser	Pro	Thr	Pro	
225					230					235					240	
aac	tcg	tcc	att	tat	gaa	acc	caa	gat	gca	aac	cta	ttt	ctt	tgt	cta	768
Asn	Cys	Ser	Ile	Tyr	Glu	Thr	Gln	Asp	Ala	Asn	Leu	Phe	Leu	Cys	Leu	
				245				250						255		
act	aaa	aac	gga	gct	cac	gta	tta	ggg	act	ata	aca	atc	aaa	ggg	ctt	816
Thr	Lys	Asn	Gly	Ala	His	Val	Leu	Gly	Thr	Ile	Thr	Ile	Lys	Gly	Leu	
			260					265					270			
aaa	gga	gca	ctg	cgg	gaa	atg	cac	gat	aac	gct	cta	tct	tta	aaa	ctt	864
Lys	Gly	Ala	Leu	Arg	Glu	Met	His	Asp	Asn	Ala	Leu	Ser	Leu	Lys	Leu	
		275					280					285				
ccc	ttt	gac	aat	cag	gga	aat	tta	ctt	aac	tgt	gcc	ttg	gaa	tca	tcc	912
Pro	Phe	Asp	Asn	Gln	Gly	Asn	Leu	Leu	Asn	Cys	Ala	Leu	Glu	Ser	Ser	
	290					295					300					
acc	tcg	cgt	tac	cag	gaa	acc	aac	gca	gtg	gcc	tct	aat	gcc	tta	aca	960
Thr	Trp	Arg	Tyr	Gln	Glu	Thr	Asn	Ala	Val	Ala	Ser	Asn	Ala	Leu	Thr	
305					310					315					320	
ttt	atg	ccc	aac	agt	aca	gtg	tat	cca	cga	aac	aaa	acc	gct	cac	ccg	1008

-40-

Phe	Met	Pro	Asn	Ser	Thr	Val	Tyr	Pro	Arg	Asn	Lys	Thr	Ala	His	Pro		
				325					330					335			
ggc	aac	atg	ctc	atc	caa	atc	tcg	cct	aac	atc	acc	ttc	agt	gtc	gtc	1056	
Gly	Asn	Met	Leu	Ile	Gln	Ile	Ser	Pro	Asn	Ile	Thr	Phe	Ser	Val	Val		
			340					345					350				
tac	aac	gag	ata	aac	agt	ggg	tat	gct	ttt	act	ttt	aaa	tgg	tca	gcc	1104	
Tyr	Asn	Glu	Ile	Asn	Ser	Gly	Tyr	Ala	Phe	Thr	Phe	Lys	Trp	Ser	Ala		
		355					360					365					
gaa	ccg	gga	aaa	cct	ttt	cac	cca	cct	acc	gct	gta	ttt	tgc	tac	ata	1152	
Glu	Pro	Gly	Lys	Pro	Phe	His	Pro	Pro	Thr	Ala	Val	Phe	Cys	Tyr	Ile		
	370					375					380						
act	gaa	gaa	taa													1164	
Thr	Glu	Glu	*														
385																	

<210> 22
 <211> 387
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ad41 short fiber

<400> 22

Met	Lys	Arg	Thr	Arg	Ile	Glu	Asp	Asp	Phe	Asn	Pro	Val	Tyr	Pro	Tyr		
1				5					10					15			
Asp	Thr	Phe	Ser	Thr	Pro	Ser	Ile	Pro	Tyr	Val	Ala	Pro	Pro	Phe	Val		
			20					25					30				
Ser	Ser	Asp	Gly	Leu	Gln	Glu	Lys	Pro	Pro	Gly	Val	Leu	Ala	Leu	Lys		
		35					40					45					
Tyr	Thr	Asp	Pro	Ile	Thr	Thr	Asn	Ala	Lys	His	Glu	Leu	Thr	Leu	Lys		
	50					55					60						
Leu	Gly	Ser	Asn	Ile	Thr	Leu	Glu	Asn	Gly	Leu	Leu	Ser	Ala	Thr	Val		
65					70				75					80			
Pro	Thr	Val	Ser	Pro	Pro	Leu	Thr	Asn	Ser	Asn	Asn	Ser	Leu	Gly	Leu		
			85					90						95			
Ala	Thr	Ser	Ala	Pro	Ile	Ala	Val	Ser	Ala	Asn	Ser	Leu	Thr	Leu	Ala		
			100					105					110				
Thr	Ala	Ala	Pro	Leu	Thr	Val	Ser	Asn	Asn	Gln	Leu	Ser	Ile	Asn	Ala		
		115					120					125					
Gly	Arg	Gly	Leu	Val	Ile	Thr	Asn	Asn	Ala	Leu	Thr	Val	Asn	Pro	Thr		
	130					135					140						
Gly	Ala	Leu	Gly	Phe	Asn	Asn	Thr	Gly	Ala	Leu	Gln	Leu	Asn	Ala	Ala		
145				150					155					160			
Gly	Gly	Met	Arg	Val	Asp	Gly	Ala	Asn	Leu	Ile	Leu	His	Val	Ala	Tyr		
			165					170						175			
Pro	Phe	Glu	Ala	Ile	Asn	Gln	Leu	Thr	Leu	Arg	Leu	Glu	Asn	Gly	Leu		
			180				185					190					
Glu	Val	Thr	Ser	Gly	Gly	Lys	Leu	Asn	Val	Lys	Leu	Gly	Ser	Gly	Leu		
		195				200						205					
Gln	Phe	Asp	Ser	Asn	Gly	Arg	Ile	Ala	Ile	Ser	Asn	Ser	Asn	Arg	Thr		
	210				215						220						
Arg	Ser	Val	Pro	Ser	Leu	Thr	Thr	Ile	Trp	Ser	Ile	Ser	Pro	Thr	Pro		
225					230					235					240		
Asn	Cys	Ser	Ile	Tyr	Glu	Thr	Gln	Asp	Ala	Asn	Leu	Phe	Leu	Cys	Leu		
				245					250					255			

-41-

Thr Lys Asn Gly Ala His Val Leu Gly Thr Ile Thr Ile Lys Gly Leu
 260 265 270
 Lys Gly Ala Leu Arg Glu Met His Asp Asn Ala Leu Ser Leu Lys Leu
 275 280 285
 Pro Phe Asp Asn Gln Gly Asn Leu Leu Asn Cys Ala Leu Glu Ser Ser
 290 295 300
 Thr Trp Arg Tyr Gln Glu Thr Asn Ala Val Ala Ser Asn Ala Leu Thr
 305 310 315 320
 Phe Met Pro Asn Ser Thr Val Tyr Pro Arg Asn Lys Thr Ala His Pro
 325 330 335
 Gly Asn Met Leu Ile Gln Ile Ser Pro Asn Ile Thr Phe Ser Val Val
 340 345 350
 Tyr Asn Glu Ile Asn Ser Gly Tyr Ala Phe Thr Phe Lys Trp Ser Ala
 355 360 365
 Glu Pro Gly Lys Pro Phe His Pro Pro Thr Ala Val Phe Cys Tyr Ile
 370 375 380
 Thr Glu Glu
 385

<210> 23
 <211> 1194
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 41sF RGD

<221> CDS
 <222> (1)... (1194)

<400> 23
 atg aaa aga acc aga att gaa gac gac ttc aac ccc gtc tac ccc tat 48
 Met Lys Arg Thr Arg Ile Glu Asp Asp Phe Asn Pro Val Tyr Pro Tyr
 1 5 10 15

 gac acc ttc tca act ccc agc atc ccc tat gta gct ccg ccc ttc gtt 96
 Asp Thr Phe Ser Thr Pro Ser Ile Pro Tyr Val Ala Pro Pro Phe Val
 20 25 30

 tct tct gac ggg tta cag gaa aaa ccc cca gga gtt tta gca ctc aag 144
 Ser Ser Asp Gly Leu Gln Glu Lys Pro Pro Gly Val Leu Ala Leu Lys
 35 40 45

 tac act gac ccc att act acc aat gct aag cat gag ctt act tta aaa 192
 Tyr Thr Asp Pro Ile Thr Thr Asn Ala Lys His Glu Leu Thr Leu Lys
 50 55 60

 ctt gga agc aac ata act tta gaa aat ggg tta ctt tcg gcc aca gtt 240
 Leu Gly Ser Asn Ile Thr Leu Glu Asn Gly Leu Leu Ser Ala Thr Val
 65 70 75 80

 ccc act gtt tct cct ccc ctt aca aac agt aac aac tcc ctg ggt tta 288
 Pro Thr Val Ser Pro Pro Leu Thr Asn Ser Asn Asn Ser Leu Gly Leu
 85 90 95

 gcc aca tcc gct ccc ata gct gta tca gct aac tct ctc aca ttg gcc 336
 Ala Thr Ser Ala Pro Ile Ala Val Ser Ala Asn Ser Leu Thr Leu Ala
 100 105 110

 acc gcc gca cca ctg aca gta agc aac aac cag ctt agt att aac gcg 384

-42-

Thr	Ala	Ala	Pro	Leu	Thr	Val	Ser	Asn	Asn	Gln	Leu	Ser	Ile	Asn	Ala		
		115					120					125					
ggc	aga	ggg	tta	gtt	ata	act	aac	aat	gcc	tta	aca	gtt	aat	cct	acc	432	
Gly	Arg	Gly	Leu	Val	Ile	Thr	Asn	Asn	Ala	Leu	Thr	Val	Asn	Pro	Thr		
		130				135					140						
gga	gcg	cta	ggg	ttc	aat	aac	aca	gga	gct	tta	caa	tta	aat	gct	gca	480	
Gly	Ala	Leu	Gly	Phe	Asn	Asn	Thr	Gly	Ala	Leu	Gln	Leu	Asn	Ala	Ala		
		145			150					155					160		
gga	gga	atg	aga	gtg	gac	ggg	gcc	aac	tta	att	ctt	cat	gta	gca	tat	528	
Gly	Gly	Met	Arg	Val	Asp	Gly	Ala	Asn	Leu	Ile	Leu	His	Val	Ala	Tyr		
				165					170					175			
ccc	ttt	gaa	gca	atc	aac	cag	cta	aca	ctg	cga	tta	gaa	aac	ggg	tta	576	
Pro	Phe	Glu	Ala	Ile	Asn	Gln	Leu	Thr	Leu	Arg	Leu	Glu	Asn	Gly	Leu		
			180					185					190				
gaa	gta	acc	agc	gga	gga	aag	ctt	aac	gtt	aag	ttg	gga	tca	ggc	ctc	624	
Glu	Val	Thr	Ser	Gly	Gly	Lys	Leu	Asn	Val	Lys	Leu	Gly	Ser	Gly	Leu		
		195				200					205						
caa	ttt	gac	agt	aac	gga	cgc	att	gct	att	agt	aat	agc	aac	cga	act	672	
Gln	Phe	Asp	Ser	Asn	Gly	Arg	Ile	Ala	Ile	Ser	Asn	Ser	Asn	Arg	Thr		
	210					215					220						
cga	agt	gta	cca	tcc	ctc	act	acc	att	tgg	tct	atc	tcg	cct	acg	cct	720	
Arg	Ser	Val	Pro	Ser	Leu	Thr	Thr	Ile	Trp	Ser	Ile	Ser	Pro	Thr	Pro		
					230				235						240		
aac	tgc	tcc	att	tat	gaa	acc	caa	gat	gca	aac	cta	ttt	ctt	tgt	cta	768	
Asn	Cys	Ser	Ile	Tyr	Glu	Thr	Gln	Asp	Ala	Asn	Leu	Phe	Leu	Cys	Leu		
				245					250					255			
act	aaa	aac	gga	gct	cac	gta	tta	ggg	act	ata	aca	atc	aaa	ggg	ctt	816	
Thr	Lys	Asn	Gly	Ala	His	Val	Leu	Gly	Thr	Ile	Thr	Ile	Lys	Gly	Leu		
		260					265						270				
aaa	gga	gca	ctg	cgg	gaa	atg	cac	gat	aac	gct	cta	tct	tta	aaa	ctt	864	
Lys	Gly	Ala	Leu	Arg	Glu	Met	His	Asp	Asn	Ala	Leu	Ser	Leu	Lys	Leu		
		275					280					285					
ccc	ttt	gac	aat	cag	gga	aat	tta	ctt	aac	tgt	gcc	ttg	gaa	tca	tcc	912	
Pro	Phe	Asp	Asn	Gln	Gly	Asn	Leu	Leu	Asn	Cys	Ala	Leu	Glu	Ser	Ser		
	290					295					300						
acc	tgg	cgt	tac	cag	gaa	acc	aac	gca	gtg	gcc	tct	aat	gcc	tta	aca	960	
Thr	Trp	Arg	Tyr	Gln	Glu	Thr	Asn	Ala	Val	Ala	Ser	Asn	Ala	Leu	Thr		
	305				310				315						320		
ttt	atg	ccc	aac	agt	aca	gtg	tat	cca	cga	aac	aaa	acc	gct	cac	ccg	1008	
Phe	Met	Pro	Asn	Ser	Thr	Val	Tyr	Pro	Arg	Asn	Lys	Thr	Ala	His	Pro		
				325				330						335			
ggc	aac	atg	ctc	atc	caa	atc	tcg	cct	aac	atc	acc	ttc	agt	gtc	gtc	1056	
Gly	Asn	Met	Leu	Ile	Gln	Ile	Ser	Pro	Asn	Ile	Thr	Phe	Ser	Val	Val		
			340				345						350				
tac	aac	gag	ata	aac	tgt	gat	tgt	cgt	ggg	gat	tgt	ttt	tgt	act	agt	1104	
Tyr	Asn	Glu	Ile	Asn	Cys	Asp	Cys	Arg	Gly	Asp	Cys	Phe	Cys	Thr	Ser		

-43-

355	360	365	
ggg tat gct ttt act ttt aaa tgg tca gcc gaa ccg gga aaa cct ttt	1152		
Gly Tyr Ala Phe Thr Phe Lys Trp Ser Ala Glu Pro Gly Lys Pro Phe			
370 375 380			
cac cca cct acc gct gta ttt tgc tac ata act gaa gaa taa	1194		
His Pro Pro Thr Ala Val Phe Cys Tyr Ile Thr Glu Glu *			
385 390 395			

<210> 24
 <211> 397
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 41sFRGD

<400> 24
 Met Lys Arg Thr Arg Ile Glu Asp Asp Phe Asn Pro Val Tyr Pro Tyr
 1 5 10 15
 Asp Thr Phe Ser Thr Pro Ser Ile Pro Tyr Val Ala Pro Pro Phe Val
 20 25 30
 Ser Ser Asp Gly Leu Gln Glu Lys Pro Pro Gly Val Leu Ala Leu Lys
 35 40 45
 Tyr Thr Asp Pro Ile Thr Thr Asn Ala Lys His Glu Leu Thr Leu Lys
 50 55 60
 Leu Gly Ser Asn Ile Thr Leu Glu Asn Gly Leu Leu Ser Ala Thr Val
 65 70 75 80
 Pro Thr Val Ser Pro Pro Leu Thr Asn Ser Asn Asn Ser Leu Gly Leu
 85 90 95
 Ala Thr Ser Ala Pro Ile Ala Val Ser Ala Asn Ser Leu Thr Leu Ala
 100 105 110
 Thr Ala Ala Pro Leu Thr Val Ser Asn Asn Gln Leu Ser Ile Asn Ala
 115 120 125
 Gly Arg Gly Leu Val Ile Thr Asn Asn Ala Leu Thr Val Asn Pro Thr
 130 135 140
 Gly Ala Leu Gly Phe Asn Asn Thr Gly Ala Leu Gln Leu Asn Ala Ala
 145 150 155 160
 Gly Gly Met Arg Val Asp Gly Ala Asn Leu Ile Leu His Val Ala Tyr
 165 170 175
 Pro Phe Glu Ala Ile Asn Gln Leu Thr Leu Arg Leu Glu Asn Gly Leu
 180 185 190
 Glu Val Thr Ser Gly Gly Lys Leu Asn Val Lys Leu Gly Ser Gly Leu
 195 200 205
 Gln Phe Asp Ser Asn Gly Arg Ile Ala Ile Ser Asn Ser Asn Arg Thr
 210 215 220
 Arg Ser Val Pro Ser Leu Thr Thr Ile Trp Ser Ile Ser Pro Thr Pro
 225 230 235 240
 Asn Cys Ser Ile Tyr Glu Thr Gln Asp Ala Asn Leu Phe Leu Cys Leu
 245 250 255
 Thr Lys Asn Gly Ala His Val Leu Gly Thr Ile Thr Ile Lys Gly Leu
 260 265 270
 Lys Gly Ala Leu Arg Glu Met His Asp Asn Ala Leu Ser Leu Lys Leu
 275 280 285
 Pro Phe Asp Asn Gln Gly Asn Leu Leu Asn Cys Ala Leu Glu Ser Ser
 290 295 300
 Thr Trp Arg Tyr Gln Glu Thr Asn Ala Val Ala Ser Asn Ala Leu Thr
 305 310 315 320

-44-

Phe	Met	Pro	Asn	Ser	Thr	Val	Tyr	Pro	Arg	Asn	Lys	Thr	Ala	His	Pro
				325					330					335	
Gly	Asn	Met	Leu	Ile	Gln	Ile	Ser	Pro	Asn	Ile	Thr	Phe	Ser	Val	Val
			340					345					350		
Tyr	Asn	Glu	Ile	Asn	Cys	Asp	Cys	Arg	Gly	Asp	Cys	Phe	Cys	Thr	Ser
		355					360					365			
Gly	Tyr	Ala	Phe	Thr	Phe	Lys	Trp	Ser	Ala	Glu	Pro	Gly	Lys	Pro	Phe
	370					375					380				
His	Pro	Pro	Thr	Ala	Val	Phe	Cys	Tyr	Ile	Thr	Glu	Glu			
385					390					395					

<210> 25
 <211> 1737
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad5 penton

<221> CDS
 <222> (1)...(1737)

<400> 25	
atg cgg cgc gcg gcg atg tat gag gaa ggt cct cct ccc tcc tac gag	48
Met Arg Arg Ala Ala Met Tyr Glu Glu Gly Pro Pro Pro Ser Tyr Glu	
1 5 10 15	
agt gtg gtg agc gcg gcg cca gtg gcg gcg gcg ctg ggt tct ccc ttc	96
Ser Val Val Ser Ala Ala Pro Val Ala Ala Ala Leu Gly Ser Pro Phe	
20 25 30	
gat gct ccc ctg gac ccg ccg ttt gtg cct ccg cgg tac ctg cgg cct	144
Asp Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro	
35 40 45	
acc ggg ggg aga aac agc atc cgt tac tct gag ttg gca ccc cta ttc	192
Thr Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe	
50 55 60	
gac acc acc cgt gtg tac ctg gtg gac aac aag tca acg gat gtg gca	240
Asp Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Thr Asp Val Ala	
65 70 75 80	
tcc ctg aac tac cag aac gac cac agc aac ttt ctg acc acg gtc att	288
Ser Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile	
85 90 95	
caa aac aat gac tac agc ccg ggg gag gca agc aca cag acc atc aat	336
Gln Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn	
100 105 110	
ctt gac gac cgg tcg cac tgg ggc ggc gac ctg aaa acc atc ctg cat	384
Leu Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His	
115 120 125	
acc aac atg cca aat gtg aac gag ttc atg ttt acc aat aag ttt aag	432
Thr Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys	
130 135 140	
gcg cgg gtg atg gtg tcg cgc ttg cct act aag gac aat cag gtg gag	480

-45-

Ala 145	Arg	Val	Met	Val	Ser 150	Arg	Leu	Pro	Thr	Lys 155	Asp	Asn	Gln	Val	Glu 160	
ctg	aaa	tac	gag	tgg	gtg	gag	ttc	acg	ctg	ccc	gag	ggc	aac	tac	tcc	528
Leu	Lys	Tyr	Glu	Trp 165	Val	Glu	Phe	Thr	Leu 170	Pro	Glu	Gly	Asn	Tyr 175	Ser	
gag	acc	atg	acc	ata	gac	ctt	atg	aac	aac	gcg	atc	gtg	gag	cac	tac	576
Glu	Thr	Met	Thr 180	Ile	Asp	Leu	Met	Asn 185	Asn	Ala	Ile	Val	Glu 190	His	Tyr	
ttg	aaa	gtg	ggc	aga	cag	aac	ggg	gtt	ctg	gaa	agc	gac	atc	ggg	gta	624
Leu	Lys	Val 195	Gly	Arg	Gln	Asn	Val 200	Val	Leu	Glu	Ser	Asp 205	Ile	Gly	Val	
aag	ttt	gac	acc	cgc	aac	ttc	aga	ctg	ggg	ttt	gac	ccc	gtc	act	ggt	672
Lys	Phe 210	Asp	Thr	Arg	Asn	Arg 215	Arg	Leu	Gly	Phe	Asp 220	Pro	Val	Thr	Gly	
ctt	gtc	atg	cct	ggg	gta	tat	aca	aac	gaa	gcc	ttc	cat	cca	gac	atc	720
Leu 225	Val	Met	Pro	Gly 230	Val	Tyr	Thr	Asn	Glu 235	Ala	Phe	His	Pro	Asp	Ile 240	
att	ttg	ctg	cca	gga	tgc	ggg	gtg	gac	ttc	acc	cac	agc	cgc	ctg	agc	768
Ile	Leu	Leu	Pro	Gly 245	Cys	Gly	Val	Asp 250	Phe	Thr	His	Ser	Arg	Leu 255	Ser	
aac	ttg	ttg	ggc	atc	cgc	aag	cgg	caa	ccc	ttc	cag	gag	ggc	ttt	agg	816
Asn	Leu	Leu 260	Gly	Ile	Arg	Lys	Arg 265	Gln	Pro	Phe	Gln	Glu	Gly 270	Phe	Arg	
atc	acc	tac	gat	gat	ctg	gag	ggt	ggt	aac	att	ccc	gca	ctg	ttg	gat	864
Ile	Thr	Tyr 275	Asp	Asp	Leu	Glu	Gly 280	Gly	Asn	Ile	Pro	Ala 285	Leu	Leu	Asp	
gtg	gac	gcc	tac	cag	gcg	agc	ttg	aaa	gat	gac	acc	gaa	cag	ggc	ggg	912
Val	Asp 290	Ala	Tyr	Gln	Ala	Ser 295	Leu	Lys	Asp	Asp	Thr 300	Glu	Gln	Gly	Gly	
ggt	ggc	gca	ggc	ggc	agc	aac	agc	agt	ggc	agc	ggc	gcg	gaa	gag	aac	960
Gly 305	Gly	Ala	Gly	Gly 310	Ser	Asn	Ser	Ser	Gly 315	Ser	Gly	Ala	Glu	Glu	Asn 320	
tcc	aac	gcg	gca	gcc	gcg	gca	atg	cag	ccg	gtg	gag	gac	atg	aac	gat	1008
Ser	Asn	Ala	Ala 325	Ala	Ala	Ala	Met	Gln 330	Pro	Val	Glu	Asp	Met 335	Asn	Asp	
agc	cgc	ggc	tac	ccc	tac	gac	gtg	ccc	gac	tac	gcg	ggc	acc	agc	gcc	1056
Ser	Arg	Gly 340	Tyr	Pro	Tyr	Asp	Val 345	Pro	Asp	Tyr	Ala	Gly 350	Thr	Ser	Ala	
aca	cgg	gct	gag	gag	aag	cgc	gct	gag	gcc	gaa	gca	gcg	gcc	gaa	gct	1104
Thr	Arg 355	Ala	Glu	Glu	Lys	Arg 360	Ala	Glu	Ala	Glu	Ala 365	Ala	Ala	Glu	Ala	
gcc	gcc	ccc	gct	gcg	caa	ccc	gag	gtc	gag	aag	cct	cag	aag	aaa	ccg	1152
Ala 370	Ala	Pro	Ala	Ala	Gln 375	Pro	Glu	Val	Glu	Lys	Pro 380	Gln	Lys	Lys	Pro	
gtg	atc	aaa	ccc	ctg	aca	gag	gac	agc	aag	aaa	cgc	agt	tac	aac	cta	1200
Val	Ile	Lys	Pro	Leu	Thr	Glu	Asp	Ser	Lys	Lys	Arg	Ser	Tyr	Asn	Leu	

-46-

385		390		395		400	
ata agc aat gac	agc acc ttc acc	cag tac cgc agc	tgg tac ctt gca	1248			
Ile Ser Asn Asp	Ser Thr Phe Thr	Gln Tyr Arg Ser	Trp Tyr Leu Ala				
	405	410	415				
tac aac tac ggc	gac cct cag acc	gga atc cgc tca	tgg acc ctg ctt	1296			
Tyr Asn Tyr Gly	Asp Pro Gln Thr	Gly Ile Arg Ser	Trp Thr Leu Leu				
	420	425	430				
tgc act cct gac	gta acc tgc ggc	tcg gag cag gtc	tac tgg tcg ttg	1344			
Cys Thr Pro Asp	Val Thr Cys	Ser Glu Gln Val	Tyr Trp Ser Leu				
	435	440	445				
cca gac atg atg	caa gac ccc gtg	acc ttc cgc tcc	acg cgc cag atc	1392			
Pro Asp Met Met	Gln Asp Pro Val	Thr Phe Arg Ser	Thr Arg Gln Ile				
	450	455	460				
agc aac ttt ccg	gtg gtg ggc gcc	gag ctg ttg ccc	gtg cac tcc aag	1440			
Ser Asn Phe Pro	Val Val Gly Ala	Glu Leu Leu Pro	Val His Ser Lys				
	465	470	475				
agc ttc tac aac	gac cag gcc gtc	tac tcc caa ctc	atc cgc cag ttt	1488			
Ser Phe Tyr Asn	Asp Gln Ala Val	Tyr Ser Gln Leu	Ile Arg Gln Phe				
	485	490	495				
acc tct ctg acc	cac gtg ttc aat	cgc ttt ccc gag	aac cag att ttg	1536			
Thr Ser Leu Thr	His Val Phe Asn	Arg Phe Pro Glu	Asn Gln Ile Leu				
	500	505	510				
gcg cgc ccg cca	gcc ccc acc atc	acc acc gtc agt	gaa aac gtt cct	1584			
Ala Arg Pro Pro	Ala Pro Thr Ile	Thr Thr Val Ser	Glu Asn Val Pro				
	515	520	525				
gct ctc aca gat	cac ggg acg cta	ccg ctg cgc aac	agc atc gga gga	1632			
Ala Leu Thr Asp	His Gly Thr Leu	Pro Leu Arg Asn	Ser Ile Gly Gly				
	530	535	540				
gtc cag cga gtg	acc att act gac	gcc aga cgc cgc	acc tgc ccc tac	1680			
Val Gln Arg Val	Thr Ile Thr Asp	Ala Arg Arg Thr	Cys Pro Tyr				
	545	550	555				
gtt tac aag gcc	ctg ggc ata gtc	tcg ccg cgc gtc	cta tcg agc cgc	1728			
Val Tyr Lys Ala	Leu Gly Ile Val	Ser Pro Arg Val	Leu Ser Ser Arg				
	565	570	575				
act ttt tga				1737			
Thr Phe *							

<210> 26
 <211> 577
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ad5 penton

<400> 26
 Arg Arg Ala Ala Met Tyr Glu Glu Gly Pro Pro Pro Ser Tyr Glu Ser

-47-

1	5	10	15
Val Val Ser Ala Pro Val Ala Ala Leu Gly Ser Pro Phe Asp			
20	25	30	
Ala Pro Leu Asp Pro Pro Phe Val Pro Pro Arg Tyr Leu Arg Pro Thr			
35	40	45	
Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe Asp			
50	55	60	
Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Thr Asp Val Ala Ser			
65	70	75	80
Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile Gln			
85	90	95	
Asn Asn Asp Tyr Ser Pro Gly Glu Ala Ser Thr Gln Thr Ile Asn Leu			
100	105	110	
Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His Thr			
115	120	125	
Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys Ala			
130	135	140	
Arg Val Met Val Ser Arg Leu Pro Thr Lys Asp Asn Gln Val Glu Leu			
145	150	155	160
Lys Tyr Glu Trp Val Glu Phe Thr Leu Pro Glu Gly Asn Tyr Ser Glu			
165	170	175	
Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile Val Glu His Tyr Leu			
180	185	190	
Lys Val Gly Arg Gln Asn Gly Val Leu Glu Ser Asp Ile Gly Val Lys			
195	200	205	
Phe Asp Thr Arg Asn Phe Arg Leu Gly Phe Asp Pro Val Thr Gly Leu			
210	215	220	
Val Met Pro Gly Val Tyr Thr Asn Glu Ala Phe His Pro Asp Ile Ile			
225	230	235	240
Leu Leu Pro Gly Cys Gly Val Asp Phe Thr His Ser Arg Leu Ser Asn			
245	250	255	
Leu Leu Gly Ile Arg Lys Arg Gln Pro Phe Gln Glu Gly Phe Arg Ile			
260	265	270	
Thr Tyr Asp Asp Leu Glu Gly Gly Asn Ile Pro Ala Leu Leu Asp Val			
275	280	285	
Asp Ala Tyr Gln Ala Ser Leu Lys Asp Asp Thr Glu Gln Gly Gly Gly			
290	295	300	
Gly Ala Gly Gly Ser Asn Ser Ser Gly Ser Gly Ala Glu Glu Asn Ser			
305	310	315	320
Asn Ala Ala Ala Ala Met Gln Pro Val Glu Asp Met Asn Asp Ser			
325	330	335	
Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Thr Ser Ala Thr			
340	345	350	
Arg Ala Glu Lys Arg Ala Glu Ala Glu Ala Ala Glu Ala Ala			
355	360	365	
Ala Pro Ala Ala Gln Pro Glu Val Glu Lys Pro Gln Lys Lys Pro Val			
370	375	380	
Ile Lys Pro Leu Thr Glu Asp Ser Lys Lys Arg Ser Tyr Asn Leu Ile			
385	390	395	400
Ser Asn Asp Ser Thr Phe Thr Gln Tyr Arg Ser Trp Tyr Leu Ala Tyr			
405	410	415	
Asn Tyr Gly Asp Pro Gln Thr Gly Ile Arg Ser Trp Thr Leu Leu Cys			
420	425	430	
Thr Pro Asp Val Thr Cys Gly Ser Glu Gln Val Tyr Trp Ser Leu Pro			
435	440	445	
Asp Met Met Gln Asp Pro Val Thr Phe Arg Ser Thr Arg Gln Ile Ser			
450	455	460	
Asn Phe Pro Val Val Gly Ala Glu Leu Leu Pro Val His Ser Lys Ser			
465	470	475	480
Phe Tyr Asn Asp Gln Ala Val Tyr Ser Gln Leu Ile Arg Gln Phe Thr			
485	490	495	

-48-

Ser Leu Thr His Val Phe Asn Arg Phe Pro Glu Asn Gln Ile Leu Ala
 500 505 510
 Arg Pro Pro Ala Pro Thr Ile Thr Thr Val Ser Glu Asn Val Pro Ala
 515 520 525
 Leu Thr Asp His Gly Thr Leu Pro Leu Arg Asn Ser Ile Gly Gly Val
 530 535 540
 Gln Arg Val Thr Ile Thr Asp Ala Arg Arg Arg Thr Cys Pro Tyr Val
 545 550 555 560
 Tyr Lys Ala Leu Gly Ile Val Ser Pro Arg Val Leu Ser Ser Arg Thr
 565 570 575
 Phe

<210> 27
 <211> 1773
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5TS35H

<221> CDS
 <222> (1)...(1773)

<400> 27
 atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gac acg gaa acc ggt cct cca act gtg cct ttt ctt act cct ccc 96
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 ttt gta tcc ccc aat ggg ttt caa gag agt ccc cct ggg gta ctc tct 144
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
 35 40 45
 ttg cgc cta tcc gaa cct cta gtt acc tcc aat ggc atg ctt gcg ctc 192
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 aaa atg ggc aac ggc ctc tct ctg gac gag gcc ggc aac ctt acc tcc 240
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 caa aat gta acc act gtg agc cca cct ctc aaa aaa acc aag tca aac 288
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
 85 90 95
 ata aac ctg gaa ata tct gca ccc ctc aca gtt acc tca gaa gcc cta 336
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
 100 105 110
 act gtg gct gcc gcc gca cct cta atg gtc gcg ggc aac aca ctc acc 384
 Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
 115 120 125
 atg caa tca cag gcc ccg cta acc gtg cac gac tcc aaa ctt agc att 432
 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
 130 135 140

-49-

gcc acc caa gga ccc ctc aca gtg tca gaa gga aag cta gcc ctg caa	480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln	
145 150 155 160	
aca tca ggc ccc ctc acc acc acc gat agc agt acc ctt act atc act	528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr	
165 170 175	
gcc tca ccc cct cta act act gcc act ggt agc ttg ggc att gac ttg	576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu	
180 185 190	
aaa gag ccc att tat aca caa aat gga aaa cta gga cta aag tac ggg	624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly	
195 200 205	
gct cct ttg cat gta aca gac gac cta aac act ttg acc gta gca act	672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr	
210 215 220	
ggt cca ggt gtg act att aat aat act tcc ttg caa act aaa gtt act	720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr	
225 230 235 240	
gga gcc ttg ggt ttt gat tca caa ggc aat atg caa ctt aat gta gca	768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala	
245 250 255	
gga gga cta agg att gat tct caa aac aga cgc ctt ata ctt gat gtt	816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val	
260 265 270	
agt tat ccg ttt gat gct caa aac caa cta aat cta aga cta gga cag	864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln	
275 280 285	
ggc cct ctt ttt ata aac tca gcc cac aac ttg gat att aac tac aac	912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn	
290 295 300	
aaa ggc ctt tac ttg ttt aca gct tca aac aat tcc aaa aag ctt gag	960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu	
305 310 315 320	
gtt aac cta agc act gcc aag ggg ttg atg ttt gac gct aca gcc ata	1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile	
325 330 335	
gcc att aat gca gga gat ggg ctt gaa ttt ggt tca cct aat gca cca	1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro	
340 345 350	
aac aca aat ccc ctc aaa aca aaa att ggc cat ggc cta gaa ttt gat	1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp	
355 360 365	
tca aac aag gct atg gtt cct aaa cta gga act ggc ctt agt ttt gac	1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp	
370 375 380	

-50-

```

agc aca ggt gcc att aca gta gga aac aaa aat aat gat aag cta act 1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385                               390                               395                               400

ttg tgg acc gga ata aac cct cca cct aac tgt caa att gtg gaa aac 1248
Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys Gln Ile Val Glu Asn
                               405                               410                               415

act aat aca aat gat ggc aaa ctt act tta gta tta gta aaa aat gga 1296
Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val Leu Val Lys Asn Gly
                               420                               425                               430

ggg ctt gtt aat ggc tac gtg tct cta gtt ggt gta tca gac act gtg 1344
Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly Val Ser Asp Thr Val
                               435                               440                               445

aac caa atg ttc aca caa aag aca gca aac atc caa tta aga tta tat 1392
Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile Gln Leu Arg Leu Tyr
                               450                               455                               460

ttt gac tct tct gga aat cta tta act gag gaa tca gac tta aaa att 1440
Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Ser Asp Leu Lys Ile
465                               470                               475                               480

cca ctt aaa aat aaa tct tct aca gcg acc agt gaa act gta gcc agc 1488
Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser Glu Thr Val Ala Ser
                               485                               490                               495

agc aaa gcc ttt atg cca agt act aca gct tat ccc ttc aac acc act 1536
Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe Asn Thr Thr
                               500                               505                               510

act agg gat agt gaa aac tac att cat gga ata tgt tac tac atg act 1584
Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile Cys Tyr Tyr Met Thr
                               515                               520                               525

agt tat gat aga agt cta ttt ccc ttg aac att tct ata atg cta aac 1632
Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile Ser Ile Met Leu Asn
                               530                               535                               540

agc cgt atg att tct tcc aat gtt gcc tat gcc ata caa ttt gaa tgg 1680
Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala Ile Gln Phe Glu Trp
545                               550                               555                               560

aat cta aat gca agt gaa tct cca gaa agc aac ata gct acg ctg acc 1728
Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn Ile Ala Thr Leu Thr
                               565                               570                               575

aca tcc ccc ttt ttc ttt tct tac att aca gaa gac gac gaa taa 1773
Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu Asp Asp Glu *
                               580                               585                               590

```

<210> 28
 <211> 590
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5TS35H

-51-

<400> 28

```

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1      5      10      15
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20      25      30
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35      40      45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50      55      60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65      70      75      80
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85      90      95
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
100      105      110
Thr Val Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
115      120      125
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
130      135      140
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145      150      155      160
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
165      170      175
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
180      185      190
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
195      200      205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
210      215      220
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225      230      235      240
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245      250      255
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260      265      270
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275      280      285
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290      295      300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305      310      315      320
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325      330      335
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340      345      350
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355      360      365
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370      375      380
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385      390      395      400
Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys Gln Ile Val Glu Asn
405      410      415
Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val Leu Val Lys Asn Gly
420      425      430
Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly Val Ser Asp Thr Val
435      440      445
Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile Gln Leu Arg Leu Tyr
450      455      460
Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu Ser Asp Leu Lys Ile
465      470      475      480

```


-52-

Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser Glu Thr Val Ala Ser
 485 490 495
 Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe Asn Thr Thr
 500 505 510
 Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile Cys Tyr Tyr Met Thr
 515 520 525
 Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile Ser Ile Met Leu Asn
 530 535 540
 Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala Ile Gln Phe Glu Trp
 545 550 555 560
 Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn Ile Ala Thr Leu Thr
 565 570 575
 Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu Asp Asp Glu
 580 585 590

<210> 29
 <211> 945
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 35TS5H

<221> CDS
 <222> (1)...(945)

<400> 29
 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta 144
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa 192
 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60
 gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa 240
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80
 aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa 288
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90 95
 cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc 336
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110
 aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag 384
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125
 gat agt att aac acc tta tgg act aca cca gct cca tct cct aac tgt 432

-53-

Asp	Ser	Ile	Asn	Thr	Leu	Trp	Thr	Thr	Pro	Ala	Pro	Ser	Pro	Asn	Cys	
	130					135					140					
aga	cta	aat	gca	gag	aaa	gat	gct	aaa	ctc	act	ttg	gtc	tta	aca	aaa	480
Arg	Leu	Asn	Ala	Glu	Lys	Asp	Ala	Lys	Leu	Thr	Leu	Val	Leu	Thr	Lys	
145					150				155						160	
tgt	ggc	agt	caa	ata	ctt	gct	aca	gtt	tca	gtt	ttg	gct	gtt	aaa	ggc	528
Cys	Gly	Ser	Gln	Ile	Leu	Ala	Thr	Val	Ser	Val	Leu	Ala	Val	Lys	Gly	
				165					170					175		
agt	ttg	gct	cca	ata	tct	gga	aca	gtt	caa	agt	gct	cat	ctt	att	ata	576
Ser	Leu	Ala	Pro	Ile	Ser	Gly	Thr	Val	Gln	Ser	Ala	His	Leu	Ile	Ile	
			180					185					190			
aga	ttt	gac	gaa	aat	gga	gtg	cta	cta	aac	aat	tcc	ttc	ctg	gac	cca	624
Arg	Phe	Asp	Glu	Asn	Gly	Val	Leu	Leu	Asn	Asn	Ser	Phe	Leu	Asp	Pro	
		195					200					205				
gaa	tat	tgg	aac	ttt	aga	aat	gga	gat	ctt	act	gaa	ggc	aca	gcc	tat	672
Glu	Tyr	Trp	Asn	Phe	Arg	Asn	Gly	Asp	Leu	Thr	Glu	Gly	Thr	Ala	Tyr	
	210					215					220					
aca	aac	gct	gtt	gga	ttt	atg	cct	aac	cta	tca	gct	tat	cca	aaa	tct	720
Thr	Asn	Ala	Val	Gly	Phe	Met	Pro	Asn	Leu	Ser	Ala	Tyr	Pro	Lys	Ser	
225					230				235						240	
cac	ggt	aaa	act	gcc	aaa	agt	aac	att	gtc	agt	caa	gtt	tac	tta	aac	768
His	Gly	Lys	Thr	Ala	Lys	Ser	Asn	Ile	Val	Ser	Gln	Val	Tyr	Leu	Asn	
				245					250					255		
gga	gac	aaa	act	aaa	cct	gta	aca	cta	acc	att	aca	cta	aac	ggt	aca	816
Gly	Asp	Lys	Thr	Lys	Pro	Val	Thr	Leu	Thr	Ile	Thr	Leu	Asn	Gly	Thr	
				260				265					270			
cag	gaa	aca	gga	gac	aca	act	cca	agt	gca	tac	tct	atg	tca	ttt	tca	864
Gln	Glu	Thr	Gly	Asp	Thr	Thr	Pro	Ser	Ala	Tyr	Ser	Met	Ser	Phe	Ser	
		275					280					285				
tgg	gac	tgg	tct	ggc	cac	aac	tac	att	aat	gaa	ata	ttt	gcc	aca	tcc	912
Trp	Asp	Trp	Ser	Gly	His	Asn	Tyr	Ile	Asn	Glu	Ile	Phe	Ala	Thr	Ser	
	290					295					300					
tct	tac	act	ttt	tca	tac	att	gcc	caa	gaa	taa						945
Ser	Tyr	Thr	Phe	Ser	Tyr	Ile	Ala	Gln	Glu	*						
305					310											

<210> 30
 <211> 314
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 35TS5H

<400> 30
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30

-54-

Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125
 Asp Ser Ile Asn Thr Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys
 130 135 140
 Arg Leu Asn Ala Glu Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys
 145 150 155 160
 Cys Gly Ser Gln Ile Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly
 165 170 175
 Ser Leu Ala Pro Ile Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile
 180 185 190
 Arg Phe Asp Glu Asn Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro
 195 200 205
 Glu Tyr Trp Asn Phe Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr
 210 215 220
 Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser
 225 230 235 240
 His Gly Lys Thr Ala Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn
 245 250 255
 Gly Asp Lys Thr Lys Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr
 260 265 270
 Gln Glu Thr Gly Asp Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser
 275 280 285
 Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser
 290 295 300
 Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu
 305 310

<210> 31
 <211> 1098
 <212> DNA
 <213> Adenovirus type 37

<220>
 <221> CDS
 <222> (1)...(1098)

<400> 31
 atg tca aag agg ctc cgg gtg gaa gat gac ttc aac ccc gtc tac ccc 48
 Met Ser Lys Arg Leu Arg Val Glu Asp Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat ggc tac gcg cgg aat cag aat atc ccc ttc ctc act ccc ccc ttt 96
 Tyr Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe
 20 25 30
 gtc tcc tcc gat gga ttc aaa aac ttc ccc cct ggg gta ctg tca ctc 144
 Val Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu
 35 40 45
 aaa ctg gct gat cca atc acc att acc aat ggg gat gta tcc ctc aag 192

-55-

Lys	Leu	Ala	Asp	Pro	Ile	Thr	Ile	Thr	Asn	Gly	Asp	Val	Ser	Leu	Lys	
	50					55					60					
gtg	gga	ggg	ggg	ctc	act	ttg	caa	gat	gga	agc	cta	act	gta	aac	cct	240
Val	Gly	Gly	Gly	Leu	Thr	Leu	Gln	Asp	Gly	Ser	Leu	Thr	Val	Asn	Pro	
65					70				75					80		
aag	gct	cca	ctg	caa	gtt	aat	act	gat	aaa	aaa	ctt	gag	ctt	gca	tat	288
Lys	Ala	Pro	Leu	Gln	Val	Asn	Thr	Asp	Lys	Lys	Leu	Glu	Leu	Ala	Tyr	
				85					90					95		
gat	aat	cca	ttt	gaa	agt	agt	gct	aat	aaa	ctt	agt	tta	aaa	gta	gga	336
Asp	Asn	Pro	Phe	Glu	Ser	Ser	Ala	Asn	Lys	Leu	Ser	Leu	Lys	Val	Gly	
			100				105						110			
cat	gga	tta	aaa	gta	tta	gat	gaa	aaa	agt	gct	gcg	ggg	tta	aaa	gat	384
His	Gly	Leu	Lys	Val	Leu	Asp	Glu	Lys	Ser	Ala	Ala	Gly	Leu	Lys	Asp	
		115					120					125				
tta	att	ggc	aaa	ctt	gtg	gtt	tta	aca	gga	aaa	gga	ata	ggc	act	gaa	432
Leu	Ile	Gly	Lys	Leu	Val	Val	Leu	Thr	Gly	Lys	Gly	Ile	Gly	Thr	Glu	
	130					135					140					
aat	tta	gaa	aat	aca	gat	ggg	agc	agc	aga	gga	att	ggg	ata	aat	gta	480
Asn	Leu	Glu	Asn	Thr	Asp	Gly	Ser	Ser	Arg	Gly	Ile	Gly	Ile	Asn	Val	
145					150					155					160	
aga	gca	aga	gaa	ggg	ttg	aca	ttt	gac	aat	gat	gga	tac	ttg	gta	gca	528
Arg	Ala	Arg	Glu	Gly	Leu	Thr	Phe	Asp	Asn	Asp	Gly	Tyr	Leu	Val	Ala	
				165					170					175		
tgg	aac	cca	aag	tat	gac	acg	cgc	aca	ctt	tgg	aca	aca	cca	gac	aca	576
Trp	Asn	Pro	Lys	Tyr	Asp	Thr	Arg	Thr	Leu	Trp	Thr	Thr	Pro	Asp	Thr	
			180				185						190			
tct	cca	aac	tgc	aca	att	gct	caa	gat	aag	gac	tct	aaa	ctc	act	ttg	624
Ser	Pro	Asn	Cys	Thr	Ile	Ala	Gln	Asp	Lys	Asp	Ser	Lys	Leu	Thr	Leu	
		195				200						205				
gta	ctt	aca	aag	tgt	gga	agt	caa	ata	tta	gct	aat	gtg	tct	ttg	att	672
Val	Leu	Thr	Lys	Cys	Gly	Ser	Gln	Ile	Leu	Ala	Asn	Val	Ser	Leu	Ile	
	210					215					220					
gtg	gtc	gca	gga	aag	tac	cac	atc	ata	aat	aat	aag	aca	aat	cca	aaa	720
Val	Val	Ala	Gly	Lys	Tyr	His	Ile	Ile	Asn	Asn	Lys	Thr	Asn	Pro	Lys	
225					230					235					240	
ata	aaa	agt	ttt	act	att	aaa	ctg	cta	ttt	aat	aag	aac	gga	gtg	ctt	768
Ile	Lys	Ser	Phe	Thr	Ile	Lys	Leu	Leu	Phe	Asn	Lys	Asn	Gly	Val	Leu	
				245					250					255		
tta	gac	aac	tca	aat	ctt	gga	aaa	gct	tat	tgg	aac	ttt	aga	agt	gga	816
Leu	Asp	Asn	Ser	Asn	Leu	Gly	Lys	Ala	Tyr	Trp	Asn	Phe	Arg	Ser	Gly	
			260					265				270				
aat	tcc	aat	gtt	tcg	aca	gct	tat	gaa	aaa	gca	att	ggg	ttt	atg	cct	864
Asn	Ser	Asn	Val	Ser	Thr	Ala	Tyr	Glu	Lys	Ala	Ile	Gly	Phe	Met	Pro	
		275				280						285				
aat	ttg	gta	gcg	tat	cca	aaa	ccc	agt	aat	tct	aaa	aaa	tat	gca	aga	912
Asn	Leu	Val	Ala	Tyr	Pro	Lys	Pro	Ser	Asn	Ser	Lys	Lys	Tyr	Ala	Arg	

-56-

290	295	300	
gac ata gtt tat gga act ata tat ctt ggt gga aaa cct gat cag cca	960		
Asp Ile Val Tyr Gly Thr Ile Tyr Leu Gly Gly Lys Pro Asp Gln Pro			
305 310 315 320			
gca gtc att aaa act acc ttt aac caa gaa act gga tgt gaa tac tct	1008		
Ala Val Ile Lys Thr Phe Asn Gln Glu Thr Gly Cys Glu Tyr Ser			
325 330 335			
atc aca ttt aac ttt agt tgg tcc aaa acc tat gaa aat gtt gaa ttt	1056		
Ile Thr Phe Asn Phe Ser Trp Ser Lys Thr Tyr Glu Asn Val Glu Phe			
340 345 350			
gaa acc acc tct ttt acc ttc tcc tat att gcc caa gaa tga	1098		
Glu Thr Thr Ser Phe Thr Phe Ser Tyr Ile Ala Gln Glu *			
355 360 365			

<210> 32
 <211> 365
 <212> PRT
 <213> Adenovirus type 37

<400> 32
 Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe
 20 25 30
 Val Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu
 35 40 45
 Lys Leu Ala Asp Pro Ile Thr Ile Thr Asn Gly Asp Val Ser Leu Lys
 50 55 60
 Val Gly Gly Gly Leu Thr Leu Gln Asp Gly Ser Leu Thr Val Asn Pro
 65 70 75 80
 Lys Ala Pro Leu Gln Val Asn Thr Asp Lys Lys Leu Glu Leu Ala Tyr
 85 90 95
 Asp Asn Pro Phe Glu Ser Ser Ala Asn Lys Leu Ser Leu Lys Val Gly
 100 105 110
 His Gly Leu Lys Val Leu Asp Glu Lys Ser Ala Ala Gly Leu Lys Asp
 115 120 125
 Leu Ile Gly Lys Leu Val Val Leu Thr Gly Lys Gly Ile Gly Thr Glu
 130 135 140
 Asn Leu Glu Asn Thr Asp Gly Ser Ser Arg Gly Ile Gly Ile Asn Val
 145 150 155 160
 Arg Ala Arg Glu Gly Leu Thr Phe Asp Asn Asp Gly Tyr Leu Val Ala
 165 170 175
 Trp Asn Pro Lys Tyr Asp Thr Arg Thr Leu Trp Thr Thr Pro Asp Thr
 180 185 190
 Ser Pro Asn Cys Thr Ile Ala Gln Asp Lys Asp Ser Lys Leu Thr Leu
 195 200 205
 Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Asn Val Ser Leu Ile
 210 215 220
 Val Val Ala Gly Lys Tyr His Ile Ile Asn Asn Lys Thr Asn Pro Lys
 225 230 235 240
 Ile Lys Ser Phe Thr Ile Lys Leu Leu Phe Asn Lys Asn Gly Val Leu
 245 250 255
 Leu Asp Asn Ser Asn Leu Gly Lys Ala Tyr Trp Asn Phe Arg Ser Gly
 260 265 270
 Asn Ser Asn Val Ser Thr Ala Tyr Glu Lys Ala Ile Gly Phe Met Pro

Asn	Leu	Val	Ala	Tyr	Pro	Lys	Pro	Ser	Asn	Ser	Lys	Lys	Tyr	Ala	Arg
Asp	Ile	Val	Tyr	Gly	Thr	Ile	Tyr	Leu	Gly	Gly	Lys	Pro	Asp	Gln	Pro
305					310					315					320
Ala	Val	Ile	Lys	Thr	Thr	Phe	Asn	Gln	Glu	Thr	Gly	Cys	Glu	Tyr	Ser
				325					330					335	
Ile	Thr	Phe	Asn	Phe	Ser	Trp	Ser	Lys	Thr	Tyr	Glu	Asn	Val	Glu	Phe
			340					345					350		
Glu	Thr	Thr	Ser	Phe	Thr	Phe	Ser	Tyr	Ile	Ala	Gln	Glu			
		355					360				365				

```
<220>
<221> CDS
<222> (1) .. (1098)
```

[illegible]

-58-

145	150	155	160		
aga gca aga gaa ggg ttg aca ttt gac aat gat gga tac ttg gta gca	Arg Ala Arg Glu Gly 165	Leu Thr Phe Asp Asn 170	Asp Gly Tyr Leu Val Ala 175	528	
tggtgg aac cca aag tat gac acg cgc aca ctt tgg aca aca cca gac aca	Trp Asn Pro Lys Tyr Asp Thr Arg 185	Leu Thr Trp Thr Thr 190	Pro Asp Thr 190	576	
tct cca aac tgc aca att gct cag gat aag gac tct aaa ctc act ttg	Ser Pro Asn Cys Thr Ile Ala Gln Asp Lys Asp Ser Lys Leu Thr Leu 195	200	205	624	
gta ctt aca aag tgt gga agt caa ata tta gct aat gtg tct ttg att	Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Asn Val Ser Leu Ile 210	215	220	672	
gtg gtc gca gga aag tac cac atc ata aat aat aag aca aat cca gaa	Val Val Ala Gly Lys Tyr His Ile Ile Asn Asn Lys Thr Asn Pro Glu 225	230	235	720	
ata aaa agt ttt act att aaa ctg tta ttt aat aag aac gga gtg ctt	Ile Lys Ser Phe Thr Ile Lys Leu Leu Phe Asn Lys Asn Gly Val Leu 245	250	255	768	
tta gac aac tca aat ctt gga aaa gct tat tgg aac ttt aga agt gga	Leu Asp Asn Ser Asn Leu Gly Lys Ala Tyr Trp Asn Phe Arg Ser Gly 260	265	270	816	
aat tcc aat gtt tcg aca gct tat gaa aaa gca att ggt ttt atg cct	Asn Ser Asn Val Ser Thr Ala Tyr Glu Lys Ala Ile Gly Phe Met Pro 275	280	285	864	
aat tta gta gcg tat cca aaa ccc agt aat tct aaa aaa tat gca aga	Asn Leu Val Ala Tyr Pro Lys Pro Ser Asn Ser Lys Lys Tyr Ala Arg 290	295	300	912	
gac ata gtt tat gga act ata tat ctt ggt gga aaa cct gat cag cca	Asp Ile Val Tyr Gly Thr Ile Tyr Leu Gly Gly Lys Pro Asp Gln Pro 305	310	315	960	
gca gtc att aaa act acc ttt aac caa gaa act gga tgt gaa tac tct	Ala Val Ile Lys Thr Thr Phe Asn Gln Glu Thr Gly Cys Glu Tyr Ser 325	330	335	1008	
atc aca ttt gac ttt agt tgg tcc aaa acc tat gaa aat gtt gaa ttt	Ile Thr Phe Asp Phe Ser Trp Ser Lys Thr Tyr Glu Asn Val Glu Phe 340	345	350	1056	
gaa acc acc tct ttt acc ttc tcc tat att gcc caa gaa tga	Glu Thr Thr Ser Phe Thr Phe Ser Tyr Ile Ala Gln Glu *	355	360	365	1098

<210> 34

<211> 365

<212> PRT

<213> Adenovirus type 19p

-59-

<400> 34

```

Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro
1      5      10      15
Tyr Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe
20      25      30
Val Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu
35      40      45
Lys Leu Ala Asp Pro Ile Thr Ile Thr Asn Gly Asp Val Ser Leu Lys
50      55      60
Val Gly Gly Gly Leu Thr Leu Gln Asp Gly Ser Leu Thr Val Asn Pro
65      70      75      80
Lys Ala Pro Leu Gln Val Thr Thr Asp Lys Lys Leu Glu Leu Ala Tyr
85      90      95
Asp Asn Pro Phe Glu Cys Ser Ala Asn Lys Phe Ser Leu Lys Val Gly
100     105     110
His Gly Leu Lys Val Leu Asp Glu Lys Ser Ala Ala Gly Leu Lys Asp
115     120     125
Leu Ile Gly Lys Leu Val Val Leu Thr Gly Lys Gly Ile Gly Thr Glu
130     135     140
Asn Leu Glu Asn Thr Asp Gly Ser Ser Arg Gly Ile Gly Ile Asn Val
145     150     155     160
Arg Ala Arg Glu Gly Leu Thr Phe Asp Asn Asp Gly Tyr Leu Val Ala
165     170     175
Trp Asn Pro Lys Tyr Asp Thr Arg Thr Leu Trp Thr Thr Pro Asp Thr
180     185     190
Ser Pro Asn Cys Thr Ile Ala Gln Asp Lys Asp Ser Lys Leu Thr Leu
195     200     205
Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Asn Val Ser Leu Ile
210     215     220
Val Val Ala Gly Lys Tyr His Ile Ile Asn Asn Lys Thr Asn Pro Glu
225     230     235     240
Ile Lys Ser Phe Thr Ile Lys Leu Leu Phe Asn Lys Asn Gly Val Leu
245     250     255
Leu Asp Asn Ser Asn Leu Gly Lys Ala Tyr Trp Asn Phe Arg Ser Gly
260     265     270
Asn Ser Asn Val Ser Thr Ala Tyr Glu Lys Ala Ile Gly Phe Met Pro
275     280     285
Asn Leu Val Ala Tyr Pro Lys Pro Ser Asn Ser Lys Lys Tyr Ala Arg
290     295     300
Asp Ile Val Tyr Gly Thr Ile Tyr Leu Gly Gly Lys Pro Asp Gln Pro
305     310     315     320
Ala Val Ile Lys Thr Thr Phe Asn Gln Glu Thr Gly Cys Glu Tyr Ser
325     330     335
Ile Thr Phe Asp Phe Ser Trp Ser Lys Thr Tyr Glu Asn Val Glu Phe
340     345     350
Glu Thr Thr Ser Phe Thr Phe Ser Tyr Ile Ala Gln Glu
355     360     365

```

<210> 35

<211> 1116

<212> DNA

<213> Adenovirus type 30

<220>

<221> CDS

<222> (1)...(1116)

<400> 35

```

atg tca aag agg ctc cgg gtg gaa gat gac ttc aac ccc gtc tac ccc
Met Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro

```

48

-60-

1	5				10				15							
tat Tyr	ggc Gly	tac Tyr	gcg Ala 20	cgg Arg	aat Asn	cag Gln	aat Asn	atc Ile 25	ccc Pro	ttc Phe	ctt Leu	act Thr	ccc Pro 30	ccc Pro	ttt Phe	96
gtc Val	tca Ser	tcc Ser 35	gat Asp	gga Gly	ttc Phe	aaa Lys	aac Asn 40	ttc Phe	cca Pro	cct Pro	ggg Gly	gtc Val 45	ctg Leu	tca Ser	ctc Leu	144
aaa Lys	ctg Leu 50	gct Ala	gac Asp	cca Pro	atc Ile	gcc Ala 55	atc Ile	act Thr	aat Asn	ggg Gly	gat Asp 60	gtc Val	tca Ser	ctc Leu	aag Lys	192
gtg Val 65	gga Gly	ggg Gly	gga Gly	cta Leu	act Thr 70	gtg Val	gaa Glu	caa Gln	gat Asp	agt Ser 75	gga Gly	aac Asn	cta Leu	agt Ser	gta Val 80	240
aac Asn	cct Pro	aag Lys	gct Ala	cca Pro 85	ttg Leu	caa Gln	gtt Val	gga Gly	aca Thr 90	gac Asp	aaa Lys	aaa Lys	ctg Leu	gaa Glu 95	ttg Leu	288
gct Ala	tta Leu	gca Ala	cct Pro 100	cca Pro	ttt Phe	gat Asp	gtc Val	aga Arg 105	gat Asp	aac Asn	aag Lys	cta Leu	gct Ala 110	att Ile	cta Leu	336
gta Val	gga Gly	gat Asp 115	gga Gly	tta Leu	aag Lys	gta Val	ata Ile 120	gat Asp	aga Arg	tca Ser	ata Ile	tct Ser 125	gat Asp	ttg Leu	cca Pro	384
ggg Gly	ttg Leu 130	tta Leu	aac Asn	tat Tyr	ctt Leu	gta Val 135	gtt Val	ttg Leu	act Thr	ggc Gly	aaa Lys 140	gga Gly	att Ile	gga Gly	aat Asn	432
gaa Glu 145	gaa Glu	tta Leu	aaa Lys	aat Asn	gac Asp 150	gat Asp	ggg Gly	agc Ser	aat Asn	aaa Lys 155	gga Gly	gtc Val	ggg Gly	tta Leu	tgt Cys 160	480
gtg Val	aga Arg	att Ile	gga Gly	gaa Glu 165	gga Gly	ggg Gly	ggg Gly	tta Leu	act Thr 170	ttt Phe	gat Asp	gat Asp	aaa Lys	ggg Gly 175	tat Tyr	528
tta Leu	gta Val	gca Ala	tgg Trp 180	aac Asn	aat Asn	aaa Lys	cat His	gac Asp 185	atc Ile	cgc Arg	aca Thr	ctt Leu	tgg Trp 190	aca Thr	act Thr	576
tta Leu	gac Asp	cct Pro 195	tct Ser	cca Pro	aat Asn	tgt Cys	aag Lys 200	ata Ile	gat Asp	ata Ile	gaa Glu	aaa Lys 205	gac Asp	tca Ser	aaa Lys	624
cta Leu	act Thr 210	ttg Leu	gta Val	ctg Leu	aca Thr	aag Lys 215	tgc Cys	gga Gly	agt Ser	cag Gln	att Ile 220	ttg Leu	gca Ala	aat Asn	gta Val	672
tct Ser 225	cta Leu	att Ile	ata Ile	gtc Val	aac Asn 230	gga Gly	aag Lys	ttc Phe	aag Lys	atc Ile 235	ctt Leu	aat Asn	aac Asn	aaa Lys	aca Thr 240	720
gac Asp	cca Pro	tcc Ser	cta Leu	cct Pro 245	aaa Lys	tca Ser	ttt Phe	aac Asn	atc Ile 250	aaa Lys	cta Leu	ctg Leu	ttt Phe	gat Asp 255	caa Gln	768

-61-

```

aat gga gtt cta ttg gaa aat tca aac att gaa aaa cag tac cta aac 816
Asn Gly Val Leu Leu Glu Asn Ser Asn Ile Glu Lys Gln Tyr Leu Asn
260 265 270

ttt aga agt gga gac tct att ctt cca gag cca tat aaa aat gca att 864
Phe Arg Ser Gly Asp Ser Ile Leu Pro Glu Pro Tyr Lys Asn Ala Ile
275 280 285

gga ttt atg cct aat tta cta gct tat gct aaa gct aca act gat cag 912
Gly Phe Met Pro Asn Leu Leu Ala Tyr Ala Lys Ala Thr Thr Asp Gln
290 295 300

tct aaa att tat gca agg aac act ata tat gga aat atc tac tta gat 960
Ser Lys Ile Tyr Ala Arg Asn Thr Ile Tyr Gly Asn Ile Tyr Leu Asp
305 310 315 320

aat cag cca tat aat cca gtt gta att aaa att act ttt aat aat gaa 1008
Asn Gln Pro Tyr Asn Pro Val Val Ile Lys Ile Thr Phe Asn Asn Glu
325 330 335

gca gat agt gct tat tct atc act ttt aac tat tca tgg acc aag gac 1056
Ala Asp Ser Ala Tyr Ser Ile Thr Phe Asn Tyr Ser Trp Thr Lys Asp
340 345 350

tat gac aat atc cct ttt gat tct act tca ttt acc ttc tcc tat atc 1104
Tyr Asp Asn Ile Pro Phe Asp Ser Thr Ser Phe Thr Phe Ser Tyr Ile
355 360 365

gcc caa gaa tga 1116
Ala Gln Glu *
370

```

```

<210> 36
<211> 370
<212> PRT
<213> Adenovirus type 30

```

```

<400> 36
Ser Lys Arg Leu Arg Val Glu Asp Asp Phe Asn Pro Val Tyr Pro Tyr
1 5 10 15
Gly Tyr Ala Arg Asn Gln Asn Ile Pro Phe Leu Thr Pro Pro Phe Val
20 25 30
Ser Ser Asp Gly Phe Lys Asn Phe Pro Pro Gly Val Leu Ser Leu Lys
35 40 45
Leu Ala Asp Pro Ile Ala Ile Thr Asn Gly Asp Val Ser Leu Lys Val
50 55 60
Gly Gly Gly Leu Thr Val Glu Gln Asp Ser Gly Asn Leu Ser Val Asn
65 70 75 80
Pro Lys Ala Pro Leu Gln Val Gly Thr Asp Lys Lys Leu Glu Leu Ala
85 90 95
Leu Ala Pro Pro Phe Asp Val Arg Asp Asn Lys Leu Ala Ile Leu Val
100 105 110
Gly Asp Gly Leu Lys Val Ile Asp Arg Ser Ile Ser Asp Leu Pro Gly
115 120 125
Leu Leu Asn Tyr Leu Val Val Leu Thr Gly Lys Gly Ile Gly Asn Glu
130 135 140
Glu Leu Lys Asn Asp Asp Gly Ser Asn Lys Gly Val Gly Leu Cys Val
145 150 155 160
Arg Ile Gly Glu Gly Gly Gly Leu Thr Phe Asp Asp Lys Gly Tyr Leu

```

[illegible]

```
<220>
<221> CDS
<222> (1) ... (1062)
```

<400> 37																
atg	gcc	aaa	cga	gct	cgg	cta	agc	agc	tcc	ttc	aat	ccg	gtc	tac	ccc	48
Met	Ala	Lys	Arg	Ala	Arg	Leu	Ser	Ser	Ser	Phe	Asn	Pro	Val	Tyr	Pro	
1				5					10					15		
tat	gaa	gat	gaa	agc	agc	tca	caa	cac	ccc	ttt	ata	aac	cct	ggg	ttc	96
Tyr	Glu	Asp	Glu	Ser	Ser	Ser	Gln	His	Pro	Phe	Ile	Asn	Pro	Gly	Phe	
			20					25					30			
att	tcc	tca	aat	ggg	ttt	gca	caa	agc	cca	gat	gga	gtt	cta	act	ctt	144
Ile	Ser	Ser	Asn	Gly	Phe	Ala	Gln	Ser	Pro	Asp	Gly	Val	Leu	Thr	Leu	
			35				40					45				
aaa	tgt	gtt	aat	cca	ctc	act	acc	gcc	agc	gga	ccc	ctc	caa	ctt	aaa	192
Lys	Cys	Val	Asn	Pro	Leu	Thr	Thr	Ala	Ser	Gly	Pro	Leu	Gln	Leu	Lys	
	50					55					60					
gtt	gga	agc	agt	ctt	aca	gta	gat	act	atc	gat	ggg	tct	ttg	gag	gaa	240
Val	Gly	Ser	Ser	Leu	Thr	Val	Asp	Thr	Ile	Asp	Gly	Ser	Leu	Glu	Glu	
65					70					75				80		
aat	ata	act	gcc	gca	gcg	cca	ctc	act	aaa	act	aac	cac	tcc	ata	ggg	288
Asn	Ile	Thr	Ala	Ala	Ala	Pro	Leu	Thr	Lys	Thr	Asn	His	Ser	Ile	Gly	

-63-

85										90					95					
tta	tta	ata	gga	tct	ggc	ttg	caa	aca	aag	gat	gat	aaa	ctt	tgt	tta	336				
Leu	Leu	Ile	Gly	Ser	Gly	Leu	Gln	Thr	Lys	Asp	Asp	Lys	Leu	Cys	Leu					
			100					105					110							
tcg	ctg	gga	gat	ggg	ttg	gta	aca	aag	gat	gat	aaa	cta	tgt	tta	tcg	384				
Ser	Leu	Gly	Asp	Gly	Leu	Val	Thr	Lys	Asp	Asp	Lys	Leu	Cys	Leu	Ser					
		115					120					125								
ctg	gga	gat	ggg	tta	ata	aca	aaa	aat	gat	gta	cta	tgt	gcc	aaa	cta	432				
Leu	Gly	Asp	Gly	Leu	Ile	Thr	Lys	Asn	Asp	Val	Leu	Cys	Ala	Lys	Leu					
	130					135					140									
gga	cat	ggc	ctt	gtg	ttt	gac	tct	tcc	aat	gct	atc	acc	ata	gaa	aac	480				
Gly	His	Gly	Leu	Val	Phe	Asp	Ser	Ser	Asn	Ala	Ile	Thr	Ile	Glu	Asn					
145					150					155					160					
aac	acc	ttg	tgg	aca	ggc	gca	aaa	cca	agc	gcc	aac	tgt	gta	att	aaa	528				
Asn	Thr	Leu	Trp	Thr	Gly	Ala	Lys	Pro	Ser	Ala	Asn	Cys	Val	Ile	Lys					
				165					170					175						
gag	gga	gaa	gat	tcc	cca	gac	tgt	aag	ctc	act	tta	gtt	cta	gtg	aag	576				
Glu	Gly	Glu	Asp	Ser	Pro	Asp	Cys	Lys	Leu	Thr	Leu	Val	Leu	Val	Lys					
			180					185					190							
aat	gga	gga	ctg	ata	aat	gga	tac	ata	aca	tta	atg	gga	gcc	tca	gaa	624				
Asn	Gly	Gly	Leu	Ile	Asn	Gly	Tyr	Ile	Thr	Leu	Met	Gly	Ala	Ser	Glu					
		195					200					205								
tat	act	aac	acc	ttg	ttt	aaa	aac	aat	caa	gtt	aca	atc	gat	gta	aac	672				
Tyr	Thr	Asn	Thr	Leu	Phe	Lys	Asn	Asn	Gln	Val	Thr	Ile	Asp	Val	Asn					
	210					215					220									
ctc	gca	ttt	gat	aat	act	ggc	caa	att	att	act	tac	cta	tca	tcc	ctt	720				
Leu	Ala	Phe	Asp	Asn	Thr	Gly	Gln	Ile	Ile	Thr	Tyr	Leu	Ser	Ser	Leu					
					230					235					240					
aaa	agt	aac	ctg	aac	ttt	aaa	gac	aac	caa	aac	atg	gct	act	gga	acc	768				
Lys	Ser	Asn	Leu	Asn	Phe	Lys	Asp	Asn	Gln	Asn	Met	Ala	Thr	Gly	Thr					
				245					250					255						
ata	acc	agt	gcc	aaa	ggc	ttc	atg	ccc	agc	acc	acc	gcc	tat	cca	ttt	816				
Ile	Thr	Ser	Ala	Lys	Gly	Phe	Met	Pro	Ser	Thr	Thr	Ala	Tyr	Pro	Phe					
			260					265					270							
ata	aca	tac	gcc	act	gag	acc	cta	aat	gaa	gat	tac	att	tat	gga	gag	864				
Ile	Thr	Tyr	Ala	Thr	Glu	Thr	Leu	Asn	Glu	Asp	Tyr	Ile	Tyr	Gly	Glu					
		275					280					285								
tgt	tac	tac	aaa	tct	acc	aat	gga	act	ctc	ttt	cca	cta	aaa	gtt	act	912				
Cys	Tyr	Tyr	Lys	Ser	Thr	Asn	Gly	Thr	Leu	Phe	Pro	Leu	Lys	Val	Thr					
	290					295					300									
gtc	aca	cta	aac	aga	cgt	atg	tta	gct	tct	gga	atg	gcc	tat	gct	atg	960				
Val	Thr	Leu	Asn	Arg	Arg	Met	Leu	Ala	Ser	Gly	Met	Ala	Tyr	Ala	Met					
					310					315					320					
aat	ttt	tca	tgg	tct	cta	aat	gca	gag	gaa	gcc	ccg	gaa	act	acc	gaa	1008				
Asn	Phe	Ser	Trp	Ser	Leu	Asn	Ala	Glu	Glu	Ala	Pro	Glu	Thr	Thr	Glu					
				325					330					335						

-64-

gtc act ctc att acc tcc ccc ttc ttt ttt tct tat atc aga gaa gat 1056
 Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp
 340 345 350

gac tga 1062
 Asp *

<210> 38
 <211> 353
 <212> PRT
 <213> Adenovirus type 16

<400> 38
 Met Ala Lys Arg Ala Arg Leu Ser Ser Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys
 50 55 60
 Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu
 65 70 75 80
 Asn Ile Thr Ala Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly
 85 90 95
 Leu Leu Ile Gly Ser Gly Leu Gln Thr Lys Asp Asp Lys Leu Cys Leu
 100 105 110
 Ser Leu Gly Asp Gly Leu Val Thr Lys Asp Asp Lys Leu Cys Leu Ser
 115 120 125
 Leu Gly Asp Gly Leu Ile Thr Lys Asn Asp Val Leu Cys Ala Lys Leu
 130 135 140
 Gly His Gly Leu Val Phe Asp Ser Ser Asn Ala Ile Thr Ile Glu Asn
 145 150 155 160
 Asn Thr Leu Trp Thr Gly Ala Lys Pro Ser Ala Asn Cys Val Ile Lys
 165 170 175
 Glu Gly Glu Asp Ser Pro Asp Cys Lys Leu Thr Leu Val Leu Val Lys
 180 185 190
 Asn Gly Gly Leu Ile Asn Gly Tyr Ile Thr Leu Met Gly Ala Ser Glu
 195 200 205
 Tyr Thr Asn Thr Leu Phe Lys Asn Asn Gln Val Thr Ile Asp Val Asn
 210 215 220
 Leu Ala Phe Asp Asn Thr Gly Gln Ile Ile Thr Tyr Leu Ser Ser Leu
 225 230 235 240
 Lys Ser Asn Leu Asn Phe Lys Asp Asn Gln Asn Met Ala Thr Gly Thr
 245 250 255
 Ile Thr Ser Ala Lys Gly Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe
 260 265 270
 Ile Thr Tyr Ala Thr Glu Thr Leu Asn Glu Asp Tyr Ile Tyr Gly Glu
 275 280 285
 Cys Tyr Tyr Lys Ser Thr Asn Gly Thr Leu Phe Pro Leu Lys Val Thr
 290 295 300
 Val Thr Leu Asn Arg Arg Met Leu Ala Ser Gly Met Ala Tyr Ala Met
 305 310 315 320
 Asn Phe Ser Trp Ser Leu Asn Ala Glu Glu Ala Pro Glu Thr Thr Glu
 325 330 335
 Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp
 340 345 350
 Asp

-65-

<210> 39
 <211> 972
 <212> DNA
 <213> Adenovirus type 35

<220>
 <221> CDS
 <222> (1)...(972)

<400> 39
 atg acc aag aga gtc cgg ctc agt gac tcc ttc aac cct gtc tac ccc 48
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt 96
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta 144
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa 192
 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60
 gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa 240
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80
 aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa 288
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90 95
 cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc 336
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110
 aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag 384
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125
 gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt 432
 Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
 130 135 140
 caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta 480
 Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
 145 150 155 160
 tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt 528
 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly
 165 170 175
 gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc 576
 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
 180 185 190

-66-

caa tta aga tta tat ttt gac tct tct gga aat cta tta act gag gaa	624
Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu	
195 200 205	
tca gac tta aaa att cca ctt aaa aat aaa tct tct aca gcg acc agt	672
Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser	
210 215 220	
gaa act gta gcc agc agc aaa gcc ttt atg cca agt act aca gct tat	720
Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr	
225 230 235 240	
ccc ttc aac acc act act agg gat agt gaa aac tac att cat gga ata	768
Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile	
245 250 255	
tgt tac tac atg act agt tat gat aga agt cta ttt ccc ttg aac att	816
Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile	
260 265 270	
tct ata atg cta aac agc cgt atg att tct tcc aat gtt gcc tat gcc	864
Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala	
275 280 285	
ata caa ttt gaa tgg aat cta aat gca agt gaa tct cca gaa agc aac	912
Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn	
290 295 300	
ata gct acg ctg acc aca tcc ccc ttt ttc ttt tct tac att aca gaa	960
Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu	
305 310 315 320	
gac gac aac taa	972
Asp Asp Asn *	

<210> 40
 <211> 323
 <212> PRT
 <213> Adenovirus type 35

<400> 40
 Met Thr Lys Arg Val Arg Leu Ser Asp Ser Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 Lys Cys Leu Thr Pro Leu Thr Thr Thr Gly Gly Ser Leu Gln Leu Lys
 50 55 60
 Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
 65 70 75 80
 Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
 85 90 95
 Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala
 100 105 110
 Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys
 115 120 125
 Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys
 130 135 140

-67-

Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val
 145 150 155 160
 Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly
 165 170 175
 Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
 180 185 190
 Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu
 195 200 205
 Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser
 210 215 220
 Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr
 225 230 235 240
 Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile
 245 250 255
 Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile
 260 265 270
 Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala
 275 280 285
 Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn
 290 295 300
 Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu
 305 310 315 320
 Asp Asp Asn

<210> 41
 <211> 1062
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad5/Ad16 chimeric fiber

<221> CDS
 <222> (1)...(1062)

<400> 41
 atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca 48
 Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 tat gaa gat gaa agc agc tca caa cac ccc ttt ata aac cct ggt ttc 96
 Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20 25 30
 att tcc tca aat ggt ttt gca caa agc cca gat gga gtt cta act ctt 144
 Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu
 35 40 45
 aaa tgt gtt aat cca ctc act acc gcc agc gga ccc ctc caa ctt aaa 192
 Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys
 50 55 60
 gtt gga agc agt ctt aca gta gat act atc gat ggg tct ttg gag gaa 240
 Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu
 65 70 75 80
 aat ata act gcc gca gcg cca ctc act aaa act aac cac tcc ata ggt 288
 Asn Ile Thr Ala Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly
 85 90 95

-68-

tta tta ata gga tct ggc ttg caa aca aag gat gat aaa ctt tgt tta	336
Leu Leu Ile Gly Ser Gly Leu Gln Thr Lys Asp Asp Lys Leu Cys Leu	
100 105 110	
tcg ctg gga gat ggg ttg gta aca aag gat gat aaa cta tgt tta tcg	384
Ser Leu Gly Asp Gly Leu Val Thr Lys Asp Asp Lys Leu Cys Leu Ser	
115 120 125	
ctg gga gat ggg tta ata aca aaa aat gat gta cta tgt gcc aaa cta	432
Leu Gly Asp Gly Leu Ile Thr Lys Asn Asp Val Leu Cys Ala Lys Leu	
130 135 140	
gga cat ggc ctt gtg ttt gac tct tcc aat gct atc acc ata gaa aac	480
Gly His Gly Leu Val Phe Asp Ser Ser Asn Ala Ile Thr Ile Glu Asn	
145 150 155 160	
aac acc ttg tgg aca ggc gca aaa cca agc gcc aac tgt gta att aaa	528
Asn Thr Leu Trp Thr Gly Ala Lys Pro Ser Ala Asn Cys Val Ile Lys	
165 170 175	
gag gga gaa gat tcc cca gac tgt aag ctc act tta gtt cta gtg aag	576
Glu Gly Glu Asp Ser Pro Asp Cys Lys Leu Thr Leu Val Leu Val Lys	
180 185 190	
aat gga gga ctg ata aat gga tac ata aca tta atg gga gcc tca gaa	624
Asn Gly Gly Leu Ile Asn Gly Tyr Ile Thr Leu Met Gly Ala Ser Glu	
195 200 205	
tat act aac acc ttg ttt aaa aac aat caa gtt aca atc gat gta aac	672
Tyr Thr Asn Thr Leu Phe Lys Asn Asn Gln Val Thr Ile Asp Val Asn	
210 215 220	
ctc gca ttt gat aat act ggc caa att att act tac cta tca tcc ctt	720
Leu Ala Phe Asp Asn Thr Gly Gln Ile Ile Thr Tyr Leu Ser Ser Leu	
225 230 235 240	
aaa agt aac ctg aac ttt aaa gac aac caa aac atg gct act gga acc	768
Lys Ser Asn Leu Asn Phe Lys Asp Asn Gln Asn Met Ala Thr Gly Thr	
245 250 255	
ata acc agt gcc aaa ggc ttc atg ccc agc acc acc gcc tat cca ttt	816
Ile Thr Ser Ala Lys Gly Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe	
260 265 270	
ata aca tac gcc act gag acc cta aat gaa gat tac att tat gga gag	864
Ile Thr Tyr Ala Thr Glu Thr Leu Asn Glu Asp Tyr Ile Tyr Gly Glu	
275 280 285	
tgt tac tac aaa tct acc aat gga act ctc ttt cca cta aaa gtt act	912
Cys Tyr Tyr Lys Ser Thr Asn Gly Thr Leu Phe Pro Leu Lys Val Thr	
290 295 300	
gtc aca cta aac aga cgt atg tta gct tct gga atg gcc tat gct atg	960
Val Thr Leu Asn Arg Arg Met Leu Ala Ser Gly Met Ala Tyr Ala Met	
305 310 315 320	
aat ttt tca tgg tct cta aat gca gag gaa gcc ccg gaa act acc gaa	1008
Asn Phe Ser Trp Ser Leu Asn Ala Glu Glu Ala Pro Glu Thr Thr Glu	
325 330 335	

-69-

```

gtc act ctc att acc tcc ccc ttc ttt ttt tct tat atc aga gaa gat 1056
Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp
                340                      345                      350

```

```

gac tga
Asp *                                     1062

```

```

<210> 42
<211> 353
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Ad5/Ad16 chimeric fiber

```

```

<400> 42
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1      5      10      15
Tyr Glu Asp Glu Ser Ser Ser Gln His Pro Phe Ile Asn Pro Gly Phe
 20      25      30
Ile Ser Ser Asn Gly Phe Ala Gln Ser Pro Asp Gly Val Leu Thr Leu
 35      40      45
Lys Cys Val Asn Pro Leu Thr Thr Ala Ser Gly Pro Leu Gln Leu Lys
 50      55      60
Val Gly Ser Ser Leu Thr Val Asp Thr Ile Asp Gly Ser Leu Glu Glu
 65      70      75      80
Asn Ile Thr Ala Ala Pro Leu Thr Lys Thr Asn His Ser Ile Gly
 85      90      95
Leu Leu Ile Gly Ser Gly Leu Gln Thr Lys Asp Asp Lys Leu Cys Leu
100      105      110
Ser Leu Gly Asp Gly Leu Val Thr Lys Asp Asp Lys Leu Cys Leu Ser
115      120      125
Leu Gly Asp Gly Leu Ile Thr Lys Asn Asp Val Leu Cys Ala Lys Leu
130      135      140
Gly His Gly Leu Val Phe Asp Ser Ser Asn Ala Ile Thr Ile Glu Asn
145      150      155      160
Asn Thr Leu Trp Thr Gly Ala Lys Pro Ser Ala Asn Cys Val Ile Lys
165      170      175
Glu Gly Glu Asp Ser Pro Asp Cys Lys Leu Thr Leu Val Leu Val Lys
180      185      190
Asn Gly Gly Leu Ile Asn Gly Tyr Ile Thr Leu Met Gly Ala Ser Glu
195      200      205
Tyr Thr Asn Thr Leu Phe Lys Asn Asn Gln Val Thr Ile Asp Val Asn
210      215      220
Leu Ala Phe Asp Asn Thr Gly Gln Ile Ile Thr Tyr Leu Ser Ser Leu
225      230      235      240
Lys Ser Asn Leu Asn Phe Lys Asp Asn Gln Asn Met Ala Thr Gly Thr
245      250      255
Ile Thr Ser Ala Lys Gly Phe Met Pro Ser Thr Thr Ala Tyr Pro Phe
260      265      270
Ile Thr Tyr Ala Thr Glu Thr Leu Asn Glu Asp Tyr Ile Tyr Gly Glu
275      280      285
Cys Tyr Tyr Lys Ser Thr Asn Gly Thr Leu Phe Pro Leu Lys Val Thr
290      295      300
Val Thr Leu Asn Arg Arg Met Leu Ala Ser Gly Met Ala Tyr Ala Met
305      310      315      320
Asn Phe Ser Trp Ser Leu Asn Ala Glu Glu Ala Pro Glu Thr Thr Glu
325      330      335
Val Thr Leu Ile Thr Ser Pro Phe Phe Phe Ser Tyr Ile Arg Glu Asp

```

-70-

Asp 340 345 350

<210> 43
 <211> 972
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad5/Ad35 chimeric fiber

<221> CDS
 <222> (1)...(972)

<400> 43

atg aag cgc gca aga ccg tct gaa gat acc ttc aac ccc gtg tat cca	48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro	
1 5 10 15	
tat gaa gat gaa agc acc tcc caa cac ccc ttt ata aac cca ggg ttt	96
Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe	
20 25 30	
att tcc cca aat ggc ttc aca caa agc cca gac gga gtt ctt act tta	144
Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu	
35 40 45	
aaa tgt tta acc cca cta aca acc aca ggc gga tct cta cag cta aaa	192
Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys	
50 55 60	
gtg gga ggg gga ctt aca gtg gat gac act gat ggt acc tta caa gaa	240
Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu	
65 70 75 80	
aac ata cgt gct aca gca ccc att act aaa aat aat cac tct gta gaa	288
Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu	
85 90 95	
cta tcc att gga aat gga tta gaa act caa aac aat aaa cta tgt gcc	336
Leu Ser Ile Gly Asn Gly Leu Glu Thr Gln Asn Asn Lys Leu Cys Ala	
100 105 110	
aaa ttg gga aat ggg tta aaa ttt aac aac ggt gac att tgt ata aag	384
Lys Leu Gly Asn Gly Leu Lys Phe Asn Asn Gly Asp Ile Cys Ile Lys	
115 120 125	
gat agt att aac acc tta tgg act gga ata aac cct cca cct aac tgt	432
Asp Ser Ile Asn Thr Leu Trp Thr Gly Ile Asn Pro Pro Pro Asn Cys	
130 135 140	
caa att gtg gaa aac act aat aca aat gat ggc aaa ctt act tta gta	480
Gln Ile Val Glu Asn Thr Asn Thr Asn Asp Gly Lys Leu Thr Leu Val	
145 150 155 160	
tta gta aaa aat gga ggg ctt gtt aat ggc tac gtg tct cta gtt ggt	528
Leu Val Lys Asn Gly Gly Leu Val Asn Gly Tyr Val Ser Leu Val Gly	
165 170 175	

-71-

```

gta tca gac act gtg aac caa atg ttc aca caa aag aca gca aac atc 576
Val Ser Asp Thr Val Asn Gln Met Phe Thr Gln Lys Thr Ala Asn Ile
      180                      185                      190

caa tta aga tta tat ttt gac tct tct gga aat cta tta act gag gaa 624
Gln Leu Arg Leu Tyr Phe Asp Ser Ser Gly Asn Leu Leu Thr Glu Glu
      195                      200                      205

tca gac tta aaa att cca ctt aaa aat aaa tct tct aca gcg acc agt 672
Ser Asp Leu Lys Ile Pro Leu Lys Asn Lys Ser Ser Thr Ala Thr Ser
      210                      215                      220

gaa act gta gcc agc agc aaa gcc ttt atg cca agt act aca gct tat 720
Glu Thr Val Ala Ser Ser Lys Ala Phe Met Pro Ser Thr Thr Ala Tyr
      225                      230                      235

ccc ttc aac acc act act agg gat agt gaa aac tac att cat gga ata 768
Pro Phe Asn Thr Thr Thr Arg Asp Ser Glu Asn Tyr Ile His Gly Ile
      245                      250                      255

tgt tac tac atg act agt tat gat aga agt cta ttt ccc ttg aac att 816
Cys Tyr Tyr Met Thr Ser Tyr Asp Arg Ser Leu Phe Pro Leu Asn Ile
      260                      265                      270

tct ata atg cta aac agc cgt atg att tct tcc aat gtt gcc tat gcc 864
Ser Ile Met Leu Asn Ser Arg Met Ile Ser Ser Asn Val Ala Tyr Ala
      275                      280                      285

ata caa ttt gaa tgg aat cta aat gca agt gaa tct cca gaa agc aac 912
Ile Gln Phe Glu Trp Asn Leu Asn Ala Ser Glu Ser Pro Glu Ser Asn
      290                      295                      300

ata gct acg ctg acc aca tcc ccc ttt ttc ttt tct tac att aca gaa 960
Ile Ala Thr Leu Thr Thr Ser Pro Phe Phe Phe Ser Tyr Ile Thr Glu
      305                      310                      315                      320

gac gac aac taa 972
Asp Asp Asn *
```

<210> 44
 <211> 323
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ad5/Ad35 chimeric fiber

```

<400> 44
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1           5           10           15
Tyr Glu Asp Glu Ser Thr Ser Gln His Pro Phe Ile Asn Pro Gly Phe
      20           25           30
Ile Ser Pro Asn Gly Phe Thr Gln Ser Pro Asp Gly Val Leu Thr Leu
      35           40           45
Lys Cys Leu Thr Pro Leu Thr Thr Gly Gly Ser Leu Gln Leu Lys
      50           55           60
Val Gly Gly Gly Leu Thr Val Asp Asp Thr Asp Gly Thr Leu Gln Glu
      65           70           75           80
Asn Ile Arg Ala Thr Ala Pro Ile Thr Lys Asn Asn His Ser Val Glu
```

-72-

				85					90					95			
Leu	Ser	Ile	Gly	Asn	Gly	Leu	Glu	Thr	Gln	Asn	Asn	Lys	Leu	Cys	Ala		
			100						105					110			
Lys	Leu	Gly	Asn	Gly	Leu	Lys	Phe	Asn	Asn	Gly	Asp	Ile	Cys	Ile	Lys		
		115					120					125					
Asp	Ser	Ile	Asn	Thr	Leu	Trp	Thr	Gly	Ile	Asn	Pro	Pro	Pro	Asn	Cys		
		130				135					140						
Gln	Ile	Val	Glu	Asn	Thr	Asn	Thr	Asn	Asp	Gly	Lys	Leu	Thr	Leu	Val		
145					150				155						160		
Leu	Val	Lys	Asn	Gly	Gly	Leu	Val	Asn	Gly	Tyr	Val	Ser	Leu	Val	Gly		
			165						170					175			
Val	Ser	Asp	Thr	Val	Asn	Gln	Met	Phe	Thr	Gln	Lys	Thr	Ala	Asn	Ile		
		180						185					190				
Gln	Leu	Arg	Leu	Tyr	Phe	Asp	Ser	Ser	Gly	Asn	Leu	Leu	Thr	Glu	Glu		
		195					200					205					
Ser	Asp	Leu	Lys	Ile	Pro	Leu	Lys	Asn	Lys	Ser	Ser	Thr	Ala	Thr	Ser		
	210					215					220						
Glu	Thr	Val	Ala	Ser	Ser	Lys	Ala	Phe	Met	Pro	Ser	Thr	Thr	Ala	Tyr		
225					230					235				240			
Pro	Phe	Asn	Thr	Thr	Thr	Arg	Asp	Ser	Glu	Asn	Tyr	Ile	His	Gly	Ile		
			245						250					255			
Cys	Tyr	Tyr	Met	Thr	Ser	Tyr	Asp	Arg	Ser	Leu	Phe	Pro	Leu	Asn	Ile		
		260					265						270				
Ser	Ile	Met	Leu	Asn	Ser	Arg	Met	Ile	Ser	Ser	Asn	Val	Ala	Tyr	Ala		
		275					280					285					
Ile	Gln	Phe	Glu	Trp	Asn	Leu	Asn	Ala	Ser	Glu	Ser	Pro	Glu	Ser	Asn		
	290				295						300						
Ile	Ala	Thr	Leu	Thr	Thr	Ser	Pro	Phe	Phe	Phe	Ser	Tyr	Ile	Thr	Glu		
305					310					315					320		
Asp	Asp	Asn															

<210> 45
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HSP binding motif

<400> 45
 Lys Lys Thr Lys
 1

<210> 46
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> conserved sequence

<400> 46
 Thr Leu Trp Thr
 1

<210> 47
 <211> 7607

-73-

<212> DNA
 <213> Artificial Sequence

<220>
 <223> GRE5-E1-SV40-Hygro

<400> 47

tctagaagat	ccgctgtaca	ggatgttcta	gctactttat	tagatccgct	gtacaggatg	60
ttctagctac	tttattagat	ccgctgtaca	ggatgttcta	gctactttat	tagatccgct	120
gtacaggatg	ttctagctac	tttattagat	ccgtgtacag	gatgtttctag	ctactttatt	180
agatcgatct	cctggccggt	cggggtcaaa	aaccagggttt	ggctataaaa	gggggtgggg	240
gcgcggttcgt	cctcactctc	ttccgcacgt	ctgtctgcga	gggccaggat	cgatcctgag	300
aacttcagggg	tgagtttggg	gacccttgat	tgttctttct	ttttcgctat	tgtaaaattc	360
atgttatatg	gagggggcaa	agttttcagg	gtgttggtta	gaatgggaag	atgtcccttg	420
tatcaccatg	gacccctcat	ataattttgt	ttctttcact	ttctactctg	ttgacaacca	480
ttgtctcctc	ttattttctt	ttcattttct	gtaacttttt	cgttaaactt	tagcttgcat	540
ttgttaacgaa	tttttaaatt	cacttttggt	tatttgtcag	attgtaagta	ctttctctaa	600
tcactttttt	ttcaaggcaa	tcagggtata	ttatatgtta	cttcagcaca	gttttagaga	660
acaattgtta	taattaaatg	ataaggtaga	atattttctgc	atataaattc	tggctggcgt	720
ggaaatatcc	ttattggtag	aaacaactac	atcctgggtca	tcacccctgcc	tttctcttta	780
tggttacaat	gatatacact	gtttgagatg	aggataaaat	actctgagtc	caaaccgggc	840
ccctctgcta	accatgttca	tgccctcttc	ttttccctac	agctcctggg	caacgtgctg	900
gttattgtgc	tgtctcatca	ttttggcaaa	gaattagatc	taagcttctg	cagctcgagg	960
actcggctga	ctgaaaatga	gacatattat	ctgccacgga	ggtgttatta	ccgaagaaat	1020
ggccgccagt	cttttggaac	agctgatcga	agaggtagct	gctgataatc	ttccacctcc	1080
tagccatttt	gaaccaccta	cccttcacga	actgtatgat	ttagacgtga	cggcccccga	1140
agatcccaac	gaggaggcgg	tttcgcagat	ttttcccgac	tctgtaatgt	tggcggtgca	1200
ggaagggatt	gacttactca	cttttccgcc	ggcgcccggt	tctccggagc	cgccctcact	1260
ttcccgccag	cccagagcag	cggagcagag	agccttgggg	ccgggtttcta	tgccaaacct	1320
tgtaccggag	gtgatcgatc	ttacctgcca	cagggtctgg	tttccaccca	gtgacgacga	1380
ggatgaagag	ggtgaggagt	ttgtgttaga	ttatgtggag	caccccgggg	acggttgacg	1440
gtcttgtcat	tatcaccgga	ggaatacggg	ggaccagat	attatgtgtt	cgctttgcta	1500
tatgaggacc	tgtggcatgt	ttgtctacag	taagtgaata	ttatgggcag	tgggtgatag	1560
agtgggtggg	ttgggtgtgt	aatttttttt	ttaattttta	cagttttgtg	gttttaagaa	1620
ttttgtattg	tgattttttt	aaaaggctct	gtgtctgaac	ctgagcctga	gcccagagcca	1680
gaaccggagc	ctgcaagacc	taccgcgcgt	cctaaaatgg	cgctgtctat	cctgagacgc	1740
ccgacatcac	ctgtgtctag	agaatgcaat	agtagtacgg	atagctgtga	ctccggtcct	1800
tctaacacac	ctcctgagat	acaccgcgtg	gtcccgctgt	gccccattaa	accagttgcc	1860
gtgagagttg	gtgggcgtcg	ccaggctgtg	gaatgtatcg	aggacttgct	taacgagcct	1920
gggcaacctt	tggactcact	ctgtaaacgc	ccaggccat	aagggtgtaa	cctgtgattg	1980
cgtgtgtggt	taacgccttt	gtttgtctga	tcaggttgatg	taagtttaat	aaagggtgag	2040
ataatgttta	acttgcacgt	cgtgttaaat	ggggcggggc	ttaaagggtg	tataatgcgc	2100
cgtgggctaa	tcttggttac	atctgacctc	atggaggctt	gggagtgttt	ggaagatttt	2160
tctgctgtgc	gtaacttgct	ggaacagagc	tctaacagta	cctcttggtt	ttggagggtt	2220
ctgtggggct	catcccaggc	aaagttagtc	tgcagaatta	aggaggatta	caagtgggaa	2280
tttgaagagc	ttttgaaatc	ctgtggtgag	ctgtttgatt	ctttgaatct	gggtcaccag	2340
gcgcttttcc	aagagaaggt	catcaagact	ttggattttt	ccacaccggg	gcgcgctgcg	2400
gctgctgttg	cttttttgag	ttttataaag	gataaatgga	gcgaagaaac	ccatctgagc	2460
gggggggtacc	tgttggaatt	tctggccatg	catctgtgga	gagcggttgt	gagacacaag	2520
aatcgccctgc	tactgttgct	ttccgtccgc	ccggcgataa	taccgacgga	ggagcagcag	2580
cagcagcagg	aggaagccag	gcggcgggcg	caggagcaga	gcccattggaa	cccgagagcc	2640
ggcctggacc	ctcggaatg	aatgttgtac	aggtggctga	actgtatcca	gaactgagac	2700
gcatttttgac	aattacagag	gatgggcagg	ggctaaaggg	ggtaaagagg	gagcgggggg	2760
cttgtgaggg	tacagaggag	gctaggaatc	tagcttttag	cttaatgacc	agacaccgtc	2820
ctgagtgtat	tacttttcaa	cagatcaagg	taaatgtcgc	taatgagctt	gatctgtctg	2880
cgcagaagta	ttccatagag	cagctgacca	cttactggct	gcagccaggg	gatgatattt	2940
aggaggctat	tagggatat	gcaaagggtg	cacttaggcc	agattgcaag	tacaagatca	3000
gcaaacttgt	aaatatcagg	aattgttgct	acatttctgg	gaacggggcc	gaggtggaga	3060
tagatacggg	ggatagggtg	gccttttagat	gtagcttagt	aaatatgtgg	ccgggggtgc	3120
ttggcatgga	cgggggtggt	attatgaatg	taagggtttac	tggccccaat	tttagcgtga	3180
cggtttttct	ggccaatacc	aaccttatcc	tacacgggtg	aagcttctat	gggtttaaca	3240

-74-

atacctgtgt	ggaagcctgg	accgatgtaa	gggttcgggg	ctgtgccttt	tactgtgtgt	3300
ggaagggggg	ggtgtgtcgc	cccaaaagca	gggcttcaat	taagaaatgc	ctctttgaaa	3360
gggtgtacct	gggtatcctg	tctgagggtg	actccagggt	gcgccacaat	gtggcctccg	3420
actgtggttg	cttcatgcta	gtgaaaagcg	tggctgtgat	taagcataac	atggatgtgt	3480
gcaactgcga	ggacagggcc	tctcagatgc	tgacctgctc	ggacggcaac	tgtcacctgc	3540
tgaagaccat	tcacgtagcc	agccactctc	gcaaggcctg	gccagtgttt	gagcataaca	3600
tactgacccg	ctgttccttg	catttgggta	acaggagggg	ggtgttccta	ccttaccaat	3660
gcaatttgag	tcacactaag	atattgcttg	agcccagag	catgtccaag	gtgaacctga	3720
acggggtgtt	tgacatgacc	atgaagatct	ggaagggtgt	gaggtacgat	gagaccgcga	3780
ccagggtgcag	accctgcgag	tgtggcggtg	aacatattag	gaaccagcct	gtgatgctgg	3840
atgtgaccga	ggagctgagg	cccgatcact	tgggtgtggc	ctgcacccgc	gctgagtttg	3900
gctctagcga	tgaagataca	gattgaggta	ctgaaatgtg	tgggcgtggc	ttaagggtgg	3960
gaaagaatat	ataaggtggg	ggtcttatgt	agttttgtat	ctgttttgca	gcagccgcgc	4020
ccgccatgag	caccaactcg	tttgatggaa	gcattgtgag	ctcataattg	acaacgcgca	4080
tgcccccatg	ggccgggggtg	cgtcagaatg	tgatgggctc	cagcattgat	ggtcgccccg	4140
tcttgcccgc	aaactctact	accttgacct	acgagaccgt	gtctggaacg	ccgttgagga	4200
ctgcagcctc	cgccgcgcgt	tcagccgctg	cagccaccgc	ccgcgggatt	gtgactgact	4260
ttgctttcct	gagcccgctt	gcaagcagtg	cagcttcccg	ttcatccgcc	cgcatgaca	4320
agttgacggc	tcttttgcca	caattggatt	ctttgacccg	ggaacttaat	gtcgtttctc	4380
agcagctgtt	ggatctgcgc	cagcaggttt	ctgccctgaa	ggcttcctcc	cctcccaatg	4440
cggtttaaaa	cataataaaa	aaaccagact	ctgtttggat	ttggatcaag	caagtgtctt	4500
gctgtctcag	ctgactgctt	aagtcgcaag	ccgaattgga	tccaattcgg	atcgatctta	4560
ttaaagcaga	acttgtttat	tgcagcttat	aatggttaca	aataaagcaa	tagcatcaca	4620
aatttcacaa	ataaagcatt	tttttctactg	cattctagtt	gtggtttgtc	caaactcatc	4680
aatgtatctt	atcatgtctg	gtcgactcta	gactcttccg	cttcctcgct	caactgactc	4740
ctgcgctcgg	tcgttcggct	gcggcgagcg	gtaacagctc	actcaaaggc	ggtaatacgg	4800
ttatccacag	aatcagggga	taacgcagga	aagaacatgt	gagcaaaagg	ccagcaaaag	4860
gccaggaacc	gtaaaaaggc	cgcgttgctg	gcgtttttcc	ataggctccg	ccccctgac	4920
gagcatcaca	aaaatcgacg	ctcaagtcag	aggtggcgaa	acccgacagg	actataaaga	4980
taccaggcgt	ttccccctgg	aagctccctc	gtgcgctctc	ctgttccgac	cctgcgcgtt	5040
accgataacc	tgtccgcctt	tctcccttcg	ggaagcgtgg	cgctttctca	tagctcacgc	5100
tgtaggtatc	tcagttcggt	gtaggtcggt	cgctccaagc	tgggctgtgt	gcacgaaccc	5160
ccggttcagc	ccgaccgctg	cgccttatcc	ggtaactatc	gtcttgagtc	caaccgggta	5220
agacacgact	tatcgccact	ggcagcagcc	actggttaaca	ggattagcag	agcgagggat	5280
gtaggcggtg	ctacagagtt	cttgaagtgg	tggcctaact	acggctacac	tagaaggata	5340
gtatttggtg	tctgcgctct	gctgaagcca	gttaccttcg	gaaaaagagt	tggtagctct	5400
tgatccggca	aacaaaccac	cgctggtagc	gggtggtttt	ttgtttgcaa	gcagcagatt	5460
acgcgcagaa	aaaaaggatc	tcaagaagat	cctttgatct	tttctacggg	gtctgacgct	5520
cagtggaaacg	aaaactcacg	ttaagggatt	ttggtcatga	gattatcaaa	aaggatcttc	5580
acctagatcc	ttttaaatga	aaaatgaatt	tctaaagtat	tctaaagtat	atatgagtaa	5640
acttggtctg	acagttacca	atgcttaatc	agtgaaggac	ctatctcagc	gatctgtcta	5700
tttcggtcat	ccatagttgc	ctgactcccc	gtcgtgtaga	taactacgat	acgggagggc	5760
ttaccatctg	gccccagtg	tgcaatgata	ccgcgagacc	cacgctcacc	ggctccagat	5820
ttatcagcaa	taaaccagcc	agccggaagg	gccgagcgca	gaagtgggtc	tgcaacttta	5880
tccgcctcca	tccagtctat	taattgttgc	cggaagccta	gagtaagtag	ttcgccagtt	5940
aatagtttgc	gcaacgttgt	tgccattgct	acaggcatcg	tgggtgtcac	ctcgtcgttt	6000
ggtatggctt	cattcagctc	cgggtcccaa	cgatcaaggc	gagttacatg	atcccccatg	6060
ttgtgcaaaa	aagcggttag	ctccttcggt	cctccgatcg	ttgtcagaag	taagttggcg	6120
gcagtgttat	cactcatggt	tatggcagca	ctgcataaatt	ctcttactgt	catgccatcc	6180
gtaagatgct	tttctgtgac	tgggtgagtag	tcaaccaagt	cattctgaga	atagtgtagt	6240
cggcgaccga	gttgctcttg	cccggcgcta	atacgggata	ataccgcgcc	acatagcaga	6300
actttaaaag	tgctcatcat	tggaaaacgt	tcttcggggc	gaaaactctc	aaggatctta	6360
ccgctgtttg	gatccagttc	gatgtaaccc	actcgtgcac	ccaactgatc	ttcagcatct	6420
ttactattca	ccagcgtttc	tgggtgagca	aaaacaggaa	ggcaaaatgc	cgcaaaaaag	6480
ggaataaggg	cgacacggaa	atggtgaata	ctcatactct	tcctttttca	atattattga	6540
agcatttatc	agggttattg	tctcatgagc	ggatacatat	ttgaatgtat	ttagaaaaat	6600
aaacaaatag	gggttccgcg	cacatttccc	cgaaaagtgc	cacctgacgt	ctaagaaacc	6660
attattatca	tgacattaac	ctataaaaaa	aggcgtatca	cgaggccctc	ttcgtctcgc	6720
gcgtttcggg	gatgacgggt	aaaacctctg	acacatgcag	ctcccggaga	cggtcacagc	6780
ttgtctgtaa	gcggatgccg	ggagcagaca	agcccgtcag	ggcgcgctcag	cgggtgttgg	6840
cgggtgtcgg	ggctggctta	actatgcggc	atcagagcag	attgtactga	gagtgcacca	6900

-75-

tatgcggtgt	gaaataccgc	acagatgcgt	aaggagaaaa	taccgcatca	ggaaattgta	6960
agcgtaaata	ttttgttaaa	attcgcgtta	aatTTTTgtt	aatcagctc	atTTTTtaac	7020
caataggccg	aaatcggcaa	aatcccttat	aaatcaaaaag	aatagaccga	gatagggttg	7080
agtgttggtc	cagtttgtaa	caagagtcca	ctattaaaga	acgtggactc	caacgtcaaa	7140
gggcgaaaaa	ccgtctatca	gggcgatggc	ccactacgtg	aaccatcacc	ctaatacaagt	7200
tttttggggg	cgaggtgccg	taaagcacta	aatcgggaacc	ctaaagggag	ccccgattt	7260
agagcttgac	ggggaaagcc	ggcgaacgtg	gcgagaaaagg	aaggggaagaa	agcgaaagga	7320
gcgggcgcta	gggcgctggc	aagtgtagcg	gtcacgctgc	gcgtaaccac	cacacccgcc	7380
gcgcttaatg	cgccgctaca	gggcgctgcc	cattcgccat	tcaggtgcg	caactgttg	7440
gaagggcgat	cgggtgcggc	ctcttcgcta	ttacgccagc	tggcgaaaagg	gggatgtgct	7500
gcaaggcgat	taagttgggt	aacgccaggg	ttttccaggt	cacgacgttg	taaaacgacg	7560
gccagtgaat	tgtaatacga	ctcactatag	ggcgaattaa	ttcgggg		7607

<210> 48

<211> 11600

<212> DNA

<213> Artificial Sequence

<220>

<223> MMTV-E2a-SV40-Neo

<400> 48

gaattccgca	ttgcagagat	attgtatttta	agtgcctagc	tcgatacaat	aaacgccatt	60
tgaccattca	ccacattggt	gtgcacctcc	aagcttgggc	agaaatggtt	gaactcccga	120
gagtgtccta	cacctagggg	agaagcagcc	aaggggttgt	ttcccaccaa	ggacgacccg	180
tctgcgcaca	aacggatgag	cccatcagac	aaagacatat	tcattctctg	ctgcaaactt	240
ggcatagctc	tgctttgcct	ggggctattg	ggggaagttg	cggttcgtgc	tcgcagggct	300
ctcacccttg	actcttttaa	tagctcttct	gtgcaagatt	acaatctaaa	caattcggag	360
aactcgacct	tcctcctgag	gcaaggacca	cagccaactt	cctcttataa	gccgcatcga	420
ttttgtcctt	cagaaataga	aataagaatg	cttgctaaaa	attatatattt	taccaataag	480
accaatccaa	taggttagatt	attagttact	atgtaaagaa	atgaatcatt	atcttttagt	540
actatTTTTa	ctcaaattca	gaagttagaa	atgggaatag	aaaatagaaa	gagacgctca	600
acctcaattg	aagaacaggt	gcaaggacta	ttgaccacag	gcctagaagt	aaaaaaggga	660
aaaaagagtg	tttttgtcaa	aataggagac	aggtgggtggc	aaccagggac	ttatagggga	720
ccttacatct	acagaccaac	agatgcccc	ttaccatata	caggaagata	tgacttaaat	780
tgggataggt	gggttacagt	caatggctat	aaagtgttat	atagatccct	cccttttctg	840
gaaagactcg	ccagagctag	acctccttgg	tgtatgttgt	ctcaagaaga	aaaagacgac	900
atgaaacaac	aggtacatga	ttatatattt	ctaggaacag	gaatgcactt	ttggggaaaag	960
attttccata	ccaaggaggg	gacagtgggt	ggactaatag	aacattattc	tgcaaaaact	1020
catggcatga	gttattatga	atagccttta	ttggcccaac	cttgcggttc	ccagggtcta	1080
agtaagtttt	tggttacaaa	ctgttcttaa	aacgaggatg	tgagacaagt	ggtttcctga	1140
cttggttttg	tatcaaaggt	tctgatctga	gctctgagtg	ttctattttc	ctatgttctt	1200
ttggaattta	tccaaatctt	atgtaaattg	ttatgtaaac	caagatataa	aagagtgtctg	1260
attttttgag	taaacttgca	acagtcctaa	cattcacctc	ttgtgtgttt	gtgtctgttc	1320
gccatcccgt	ctccgctcgt	cacttatcct	tcactttcca	gaggggtccc	ccgcagacct	1380
cggcgaccct	caggctcgcc	gactgcggca	gctggcgccc	gaacagggac	cctcggataa	1440
gtgacccttg	tctctatttc	tactatttgg	tgtttgtcct	gtattgtctc	tttcttgtct	1500
ggctatcatc	acaagagcgg	aacggactca	ccatagggac	caagctagcg	cttctcgtcg	1560
cgtccaagac	cctcaaagat	ttttggcact	tcgttgagcg	aggcgatatc	aggtatgaca	1620
gcgccttgcc	gcaaggccag	ctgcttgtcc	gctcggtgc	ggttggcacg	gcaggatagg	1680
ggtatcttgc	agttttgtaa	aaagatgtga	taggtggcaa	gcacctctgg	cacggcaaat	1740
acggggtaga	agttagggcg	cgggttgggc	tcgcatgtgc	cgttttcttg	gcgtttgggg	1800
ggtacgcgcg	gtgagaatag	gtggcggttcg	taggcaaggc	tgacatccgc	tatggcgagg	1860
ggcacatcgc	tgcgctcttg	caacgcgtcg	cagataatgg	cgactggcg	ctgcagatgc	1920
ttcaacagca	cgtcgtctcc	cacatctagg	tagtcgccat	gcctttcgtc	ccccgcgccg	1980
acttgttcct	cgtttgcctc	tgcgttgttc	tgggtcttgc	ttttatcctc	tgttgggtact	2040
gagcgggtcct	cgtcgtcttc	gcttacaaaa	cctgggtcct	gctcgataat	cacttcctcc	2100
tcctcaagcg	ggggtgcctc	gacggggaag	gtggtaggcg	cgttggcggc	atcgggtggag	2160
cgggtgggtg	cgaactcaga	gggggcgggt	aggctgtcct	tcttctcgac	tgactccatg	2220
atctttttct	gcctatagga	gaaggaaatg	gccagtcggg	aagaggagca	gcgcgaaacc	2280
acccccgagc	gcggacgcgg	tgcggcgcca	cgtcccccaa	ccatggagga	cgtgtcgtcc	2340

-76-

ccgtccccgt	cgccgcccgc	tccccgggcg	cccccaaaaa	agcggatgag	gcggcgatc	2400
gagtcgag	acgaggaaga	ctcatcacia	gacgcgctgg	tgccgcgcac	accagccccg	2460
cgcccatcga	cctcggcggc	ggatttggcc	attgcgccc	agaagaaaaa	gaagcgccct	2520
tctcccaagc	ccgagcgccc	gccatcacca	gaggtaatcg	tggaacagcga	ggaagaaaga	2580
gaagatgtgg	cgctacaaat	ggtgggtttc	agcaaccac	cggtgctaata	caagcatggc	2640
aaaggaggta	agcgcacagt	gcgggcggtg	aatgaagacg	accagtggtg	gcgtggtatg	2700
cggacgcaag	aggaagagga	agagcccagc	gaagcggaaa	gtgaaattac	ggtgatgaac	2760
ccgctgagtg	tgccgatcgt	gtctgcgtgg	gagaagggca	tggaaggctgc	gcgcgcgctg	2820
atggacaagt	accacgtgga	taacgatcta	aaggcgaact	tcaaactact	gcctgaccaa	2880
gtggaagctc	tggcggccgt	atgcaagacc	tggtgaaacg	aggagcacccg	cggtgtgcag	2940
ctgaccttca	ccagcaacaa	gacctttgtg	acgatgatgg	ggcgattcct	gcaggcgctac	3000
ctgcagtcgt	ttgcagaggt	gacctacaag	catcacgagc	ccacgggctg	cgctgtgtgg	3060
ctgcaccgct	gcgctgagat	cgaaggcgag	cttaagtgtc	tacacggaag	cattatgata	3120
aataaggagc	acgtgattga	aatggatgtg	acgagcgaaa	acgggcagcg	cgcgctgaag	3180
gagcagtcct	gcaaggccaa	gatcgtgaag	aacgggtggg	gcccgaatgt	ggtgcagatc	3240
tccaacaccg	acgcaagggt	ctgcgtgcac	gacgcggcct	gtccggccaa	tcagttttcc	3300
ggcaagtcct	gcggcatggt	cttctctgaa	ggcgcaaaag	ctcaggtggc	ttttaagcag	3360
atcaaggctt	ttatgcaggc	gctgtatcct	aacgcccaga	ccgggcacgg	tcaccttttg	3420
atgccactac	ggtgcgagtg	caactcaaag	cctgggcacg	cgcccttttt	gggaaggcag	3480
ctaccaaaag	tgactccgtt	cgccctgagc	aacgcggagg	acctggacgc	ggatctgatc	3540
tccgacaaga	gcgtgctggc	cagcgtgcac	caccgcggcg	tgatagtgtt	ccagtgtgc	3600
aaccctgtgt	atcgcaactc	gcgcgcgcag	ggcgagggcc	ccaactgcga	cttcaagata	3660
toggcgcccc	acctgctaaa	cgcgttgggtg	atggtgcgca	gcctgtggag	tgaaaacttc	3720
accgagctgc	cgcgatgggt	tgtgcctgag	tttaagtggg	gcactaaaca	ccagtatcgc	3780
aacgtgtccc	tgccagtggc	gcatagcgat	gcgcggcaga	accccttttg	tttttaaagc	3840
gcgcagacgg	caagggtggg	ggtaaaataat	cacccgagag	tgtacaaata	aaagcatttg	3900
cctttattga	aagtgtctct	agtacattat	ttttacatgt	ttttcaagtg	acaaaaagaa	3960
gtggcgctcc	taatctgcgc	actgtggctg	cggaagttagg	gcgagtggcg	ctccaggaag	4020
ctgtagagct	gttcctgggt	gcgacgcagg	gtgggctgta	cctggggact	ggtgagcatg	4080
gagttgggta	ccccggtaat	aagggttcag	gtggggttgt	gatccatggg	agtttggggc	4140
cagttggcaa	aggcgtggag	aaacatgcag	cagaatagtc	cacaggcggc	cgagttgggc	4200
ccctgtacgc	tttgggtgga	cttttcagc	gttatacagc	ggtcggggga	agaagcaatg	4260
gcgctacggc	gcaggagtga	ctcgtactca	aactggtaaa	cctgcttgag	tcgctggtca	4320
gaaaagccaa	agggctcaaa	gaggtagcat	gtttttgagt	gcgggttcca	ggcaaaggcc	4380
atccagtgtg	cgccccagc	ctcgcgaccg	gccgtattga	ctatggcgca	ggcgagcttg	4440
tgtggagaaa	caaagcctgg	aaagcgcttg	tcataagggtg	ccaaaaata	tgggcccaa	4500
ccaagatctt	tgacaatggc	tttcagttcc	tgctcactgg	agcccatggc	ggcagctgtt	4560
gttgatgttg	cttgcttctt	tatgttgttg	cgttgocggc	cgagaagggc	gtgcgcaggt	4620
acacggtttc	gatgacgccg	cggtgcggcc	ggtgcacacg	gaccacgtca	aagacttcaa	4680
acaaaacata	aagaagggtg	ggctcgtcca	tggtgacctc	atataggggc	cggttgctaa	4740
ttacctcagg	tcgacctcga	gggatctttg	tgaaggaacc	ttacttctgt	ggtgtgacat	4800
aattggacaa	actacctaca	gagattttaa	gctctaagg	aaatataaaa	tttttaagt	4860
tataatgtgt	taaactactg	attctaattg	tttgtgtatt	ttagattcca	acctatggaa	4920
ctgatgaatg	ggagcagtg	tggaaatgct	ttaatgagga	aaacctgttt	tgctcagaag	4980
aatgccatc	tagtgatgat	gaggctactg	ctgactctca	acattctact	cctccaaaaa	5040
agaagagaaa	ggtagaagac	cccaaggact	ttccttcaga	attgctaagt	tttttgagtc	5100
atgctgtgtt	tagtaataga	actcttgctt	gctttgctat	ttacaccaca	aaggaaaaag	5160
ctgcactgct	atacaagaaa	attatggaaa	aatattctgt	aacctttata	agtaggcata	5220
acagttataa	tcataacata	ctgttttttc	ttactccaca	caggcataga	gtgtctgcta	5280
ttataaacta	tgctcaaaaa	ttgtgtacct	ttagcttttt	aatttgtaaa	gggttgtaaa	5340
aggaatatatt	gatgtatagt	gccttgacta	gagatcataa	tcagccatac	cacatttgta	5400
gaggttttac	ttgcttttaa	aaacctccca	cacctcccc	tgaacctgaa	acataaaatg	5460
aatgcaattg	ttgtgtttaa	cttggtttat	gcagcttata	atggttacaa	ataaagcaat	5520
agcatcacia	atttcacaaa	taaagcattt	tttccactgc	attctagtgt	tggtttgtcc	5580
aaactcatca	atgtatctta	tcatgtctgg	atccggctgt	ggaatgtgtg	tcagttaggg	5640
tgtggaaagt	cccaggctc	cccagcaggc	agaagtatgc	aaagcatgca	tctcaattag	5700
tcagcaacca	ggtgtggaaa	gtccccaggc	tccccagcag	gcagaagtat	gcaaagcatg	5760
cactccaatt	agtcagcaac	catagctccc	cccctaactc	cgcccatccc	gcccctaact	5820
ccgcccagtt	ccgcccattc	tcgccccatc	ggctgactaa	ttttttttat	ttatgcagag	5880
gccgaggccg	cctcggcctc	tgagctattc	cagaagttagt	gaggaggctt	ttttggaggc	5940
ctaggctttt	gcaaaaagct	tcacgctgcc	gcaagcactc	agggcgcaag	ggctgctaaa	6000

-77-

ggaagcggaa	cacgtagaaa	gccagtcg	agaaacgg	ctgaccccg	atgaatgtca	6060
gctactggg	tatctggaca	agggaaaac	caagcgcaaa	gagaaagcag	gtagctttgca	6120
gtgggcttac	atggcgatag	ctagactggg	cgggttttatg	gacagcaagc	gaaccgggaat	6180
tgccagctgg	ggcgccctct	ggtaagggtt	ggaagccctg	caaagtaa	tggatggctt	6240
tcttgccgcc	aaggatctga	tggcgaggg	gatcaagatc	tgatcaagag	acaggatgag	6300
gatcgtttcg	catgattgaa	caagatggat	tgcacgcagg	ttctccggcc	gcttgggttg	6360
agaggctatt	cggctatgac	tgggcacaac	agacaatcgg	ctgctctgat	gccgccgtgt	6420
tccggctgtc	agcgagggg	cgcccggttc	tttttgtcaa	gaccgacctg	tccgggtgcc	6480
tgaatgaact	gcaggacgag	gcagcgcggc	tatcgtggct	ggccacgacg	ggcgttcctt	6540
gcgcagctgt	gctcgacgtt	gtcactgaag	cgggaagggg	ctggctgcta	ttgggcgaag	6600
tgccggggga	ggatctcctg	tcactctcacc	ttgctcctgc	cgagaaagta	tccatcatgg	6660
ctgatgcaat	gcggcggtcg	catacgcttg	atccggctac	ctgcccattc	gaccaccaag	6720
cgaaacatcg	catcgagcga	gcacgtactc	ggatggaagc	cggctctgtc	gatcaggatg	6780
atctggacga	agagcatcag	gggctcgcgc	cagccgaact	gttcgccagg	ctcaaggcgc	6840
gcatgcccg	cggcgaggat	ctcgtcgtga	cccatggcga	tgcttgcctg	ccgaatatca	6900
tggtggaaaa	tggccgcttt	tctggattca	tcgactgtgg	cgggctgggt	gtggcggacc	6960
gctatcagga	catagcgttg	gctaccgtg	atattgctga	agagcttggc	ggcgaatggg	7020
ctgaccgctt	cctcgtgctt	tacgggtatcg	ccgctcccg	ttcgacgcgc	atcgcttctt	7080
atcgccctct	tgacgagttc	ttctgagcgg	gactctgggg	ttcgaaatga	ccgaccaagc	7140
gacgcccaac	ctgccatcac	gagatttcga	ttccaccgcc	gccttctatg	aaagggtggg	7200
cttcggaatc	gttttcggg	acgccggctg	gatgatcctc	cagcgcgggg	atctcatgct	7260
ggagttcttc	gcccaccccg	ggctcgatcc	cctcgcgagt	tgggttcagct	gctgcctgag	7320
gctggacgac	ctcgcgaggt	tctaccggca	gtgcaaatcc	gtcggcatcc	aggaaaccag	7380
cagcggtcat	ccgcgcaccc	atgcccccg	actgcaggag	tggggaggca	cgatggccgc	7440
tttgggtccc	gatccttgtg	aaggaacctt	acttctgtgg	tgtgacataa	ttggacaaa	7500
tacctacaga	gatttaaagc	tctaaggtaa	atataaaatt	tttaagtgtg	taatgtgtta	7560
aactactgat	tctaattggt	tgtgtatatt	agattccaac	ctatggaact	gatgaatggg	7620
agcagtgggt	gaatgccttt	aatgaggaaa	acctgttttg	ctcagaagaa	atgccatcta	7680
gtgatgatga	ggctactgct	gactctcaac	attctactcc	tccaaaaaag	aagagaaagg	7740
tagaagaccc	caaggacttt	ccttcagaat	tgctaagtgt	tttgagtcat	gctgtgttta	7800
gtaatagAAC	tcttgcttgc	tttgctatatt	acaccacaaa	ggaaaaagct	gcactgctat	7860
acaagaaaat	tatggaaaaa	tattctgtaa	cctttataag	taggcataac	agttataatc	7920
ataacatact	gttttttctt	actccacaca	ggcatagagt	gtctgctatt	aataactatg	7980
ctcaaaaaat	gtgtaccttt	agctttttta	tttgtaaagg	ggttaataag	gaatatttga	8040
tgtatagtgc	cttgataga	gatcataatc	agccatacca	catttgtaga	ggttttactt	8100
gctttaaaaa	acctcccaga	cctccccctg	aacctgaaac	ataaaatgaa	tgcaattgtt	8160
gttggttaact	tgtttattgc	agcttataat	ggttacaaat	aaagcaatag	catcacaat	8220
ttcacaaaata	aagcattttt	ttcactgcat	tctagtgtgt	gtttgtccaa	actcatcaat	8280
gtatcttatc	atgtctggat	ccccaggaag	ctcctctgtg	tcctcataaa	ccctaacctc	8340
ctctacttga	gaggacattc	caatcatagg	accctctgtg	accctctgtg	tcctctctgt	8400
aattaggtca	cttaacaaaa	aggaaattgg	gtaggggttt	ttcacagacc	gcttttctaag	8460
ggtaatttta	aaatatctgg	gaagtccctt	ccactgctgt	gttcagaaag	tggttggttaa	8520
cagcccacaa	atgtcaacag	cagaaacata	caagctgtca	gctttgcaca	agggcccaac	8580
accctgctca	tcaagaagca	ctgtggttgc	tgtgttagta	atgtgcaaaa	caggaggcac	8640
atttttcccca	cctgtgtagg	ttccaaaata	tctagtgttt	tcattttttac	ttggatcagg	8700
aaccagcac	tccactggat	aagcattatc	cttatccaaa	acagccttgt	ggtcagtgtt	8760
catctgctga	ctgtcaactg	tagcattttt	tgggggttaca	gtttgagcag	gatattttgg	8820
cctgtagttt	gctaacacac	cctgcagctc	caaagggttc	ccaccaacag	caaaaaatg	8880
aaaatttgac	ccttgaatgg	gttttccagc	accattttca	tgagtttttt	gtgtccctga	8940
atgcaagttt	aacatagcag	ttaccccaat	aacctcagtt	ttaacagtaa	cagcttccca	9000
catcaaaata	tttccacagg	ttaagtcctc	atttaaatta	ggcaaaggaa	ttcttgaaga	9060
cgaaagggcc	tcgtgatacg	cctattttta	taggttaatg	tcatagataat	aatggtttct	9120
tagacgtcag	gtggcacttt	tccgggaaat	gtgcgcggaa	cccctatttg	tttatttttc	9180
taaatacatt	caaatatgta	tccgctcatg	agacaataac	cctgataaat	gcttcaataa	9240
tattgaaaaa	ggaagagtat	gagtattcaa	catttccgtg	tcgcccttat	tccttttttt	9300
gcggcatttt	gccttcctgt	ttttgctcac	ccagaaacgc	tggtgaaagt	aaaagatgct	9360
gaagatcagt	tgggtgcacg	agtgggttac	atcgaaactg	atctcaacag	cggttaagatc	9420
cttgagagtt	ttcgccccga	agaacgtttt	ccaatgatga	gcacttttta	agttctgtcta	9480
tgtggcgcg	tattatcccg	tggtgacgcc	gggaagagc	aactcggtcg	ccgcatacac	9540
tattctcaga	atgacttggg	tgagtactca	ccagtcacag	aaaagcatct	tacggatggc	9600
atgacagtaa	gagaattatg	cagtgcgtgc	ataaccatga	gtgataaac	tgccggccaac	9660

-78-

```

ttactttctga caacgatcgg aggaccgaag gagctaaccg ctttttttgca caacatgggg 9720
gatcatgtaa ctgcgcttga tcgttgggaa cgggagctga atgaagccat accaaacgac 9780
gagcgtgaca ccacgatgcc tgcagcaatg gcaacaacgt tgcgcaaact attaaactggc 9840
gaactactta ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt 9900
gcaggaccac ttctgcgctc ggcccttccg gctggctggt ttattgctga taaatctgga 9960
gccggtgagc gtgggtctcg cggatcatt gcagcactgg ggccagatgg taagccctcc 10020
cgtatcgtag ttatctacac gacggggagt caggcaacta tggatgaacg aaatagacag 10080
atcgcctgaga taggtgcctc actgattaag cattggtaac tgtcagacca agtttactca 10140
tatatacttt agattgattt aaaacttcat ttttaattta aaaggatcta ggtgaagatc 10200
cttttttgata atctcatgac caaaatccct taacgtgagt ttctgttcca ctgagcgtca 10260
gaccccgtag aaaagatcaa aggatcttct tgagatcctt tttttctgcg cgtaatctgc 10320
tgcttgcaaa caaaaaaacc accgctacca gcggtggttt gtttgccgga tcaagagcta 10380
ccaactcttt ttccgaaggt aactggcttc agcagagcgc agataccaaa tactgtcctt 10440
ctagtgtagc cgtagttagg ccaccacttc aagaactctg tagcaccgcc tacatacctc 10500
gctctgctaa tcctgttacc agtggctgct gccagtggcg ataagtcgtg tcttaccggg 10560
ttggactcaa gacgatagtt accggataag gcgcagcggg cgggctgaac ggggggttcg 10620
tgcacacagc ccagcttgga gcgaacgacc tacaccgaac tgagatacct acagcgtgag 10680
ctatgagaaa gcgccacgct tccgaaggg agaaaggcgg acaggatatcc ggtaagcggc 10740
agggctcgaa caggagagcg cacgaggag cttccagggg gaaacgcctg gtatctttat 10800
agtcctgtcg ggtttcgcca cctctgactt gacgctcgat ttttgtgatg ctgcgtcagg 10860
gggcggagcc tatggaaaaa cgccagcaac gcggcctttt tacggttcctt ggccttttgc 10920
tggccttttg ctcacatggt ctttcctgcg ttatccctg attctgtgga taaccgtatt 10980
accgcctttg agtgagctga taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca 11040
gtgagcgagg aagcggaaga gcgcctgat cggtattttc tccttacgca tctgtgcggt 11100
atttcacacc gcatatggtg cactctcagt acaatctgct ctgatgccgc atagttaagc 11160
cagtatctgc tccctgcttg tgtgttgag gtcgctgagt agtgcgcgag caaaatttaa 11220
gctacaacaa ggcaaggctt gaccgacaat tgcatgaaga atctgcttag ggtaggcgt 11280
tttgcgctgc ttcgcgatgt acgggccaga tatacgcgta tctgagggga ctagggtgtg 11340
tttagcgaa aagcggggct tcggttgtag gcggttagga gtccctcag gatatagtag 11400
tttcgctttt gcatagggag ggggaaatgt agtcttatgc aatacacttg tagtcttgca 11460
acatggtaac gatgagttag caacatgcct tacaaggaga gaaaaagcac cgtgcatgcc 11520
gattggtgga agtaagggtg tacgatcgtg ctttattagg aaggcaacag acgggtctga 11580
catggattgg acgaaccact

```

<210> 49

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Ad5 penton residues 337-344

<400> 49

His Ala Ile Arg Gly Asp Thr Phe

1

5

<210> 50

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> PD1 penton mutation

<400> 50

Ser Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Thr Ser

1

5

10

15

<210> 51

-79-

<211> 35211

<212> DNA

<213> PArtificial Sequence

<220>

<223> Plasmid Av1nBg

<400> 51

catcatcaat	aatatacctt	atthttggatt	gaagccaata	tgataatgag	ggggtggagt	60
ttgtgacgtg	gcgcggggcg	tgggaacggg	gcgggtgacg	tagtagtggtg	gcggaagtgt	120
gatgttgcaa	gtgtggcgga	acacatgtaa	gcgacggatg	tggcaaaagt	gacgtttttg	180
gtgtgcgcgc	gtgtacacag	gaagtgacaa	ttttcgcgcg	gttttaggcg	gatgtttag	240
taaatttggg	cgtaaccgag	taagatttgg	ccattttcgc	gggaaaactg	aataagagga	300
agtgaatct	gaataattht	gtgttactca	tagcgcgtaa	tatttgtcta	gggccgcggg	360
gactttgacc	gtttacgtgg	agactcgccc	aggtgttttt	ctcaggtgtt	ttccgcgttc	420
cgggtcaaag	ttggcgtttt	attattatag	tcagtacgta	ccagtgcact	ggcctaggaa	480
gcttggtacc	ggtgaattcg	ctagcgttcg	cgccccgatg	tacgggccag	atatacgctg	540
atctgagggg	actaggggtg	gtttaggcga	aaagcggggc	ttcggttgta	cgcggttagg	600
agtccctca	ggatatagta	gtttcgcttt	tgcataggga	gggggaaatg	tagtcttatg	660
caatactctt	gtagtcttgc	aacatggtaa	cgatgagtta	gcaacatgcc	ttacaaggag	720
agaaaaagca	ccgtgcctgc	cgattgggtg	aagtaagggtg	gtacgatcgt	gccttattag	780
gaaggcaaca	gacgggtctg	acatggattg	gacgaaccac	tgaattccgc	attgcagaga	840
tattgtatth	aagtgcctag	ctcgatacaa	taaaccgcat	ttgaccattc	accacatttg	900
tgtgcacctc	cggccctggc	cactctcttc	cgcatcgctg	tctgcggggg	ccagctgttg	960
ggctcgcggg	tgaggacaaa	ctcttcgcgg	tctttccagt	actcttggat	cggaaaacctg	1020
tcggcctccg	aacgggtact	cgccgcgag	ggacctgagc	gagtcgcac	cgaccggatc	1080
ggaaaaacct	tcgagaaagg	cgtgtaacca	gtcacagtcg	ctctagaact	agtggatccc	1140
ccgggctgca	ggaattcgat	ctagatggat	aaaggtccaa	aaaagaagag	aaaggtagaa	1200
gaccccaagg	actttccttc	agaattgcta	agttttttga	gtgatccact	ggccgtcgtt	1260
ttacaacgtc	gtgactggga	aaaccctggc	gttacccaac	ttaatcgctt	tgcagcacat	1320
ccccctttcg	ccagctggcg	taatagcgaa	gagggccgca	ccgatcgccc	ttcccaacag	1380
ttgcgcagcc	tgaatggcga	atggcgcttt	gcctggtttc	cggcaccaga	agcggtgccg	1440
gaaagctggc	tggagtgcga	tcttcctgag	gccgatactg	tcgtcgtccc	ctcaaactgg	1500
cagatgcacg	gttacgatgc	gcccactctac	accaacgtaa	cctatcccac	tacggtcaat	1560
ccgcggtttg	ttcccacgga	gaatccgacg	ggttggttact	cgctcacatt	taatgttgat	1620
gaaagctggc	tacaggaagg	ccagacgcga	attatttttg	atggcgttaa	ctcggcgttt	1680
catctgtggt	gcaacggggc	ctgggtcggg	tacggccagg	acagtcgttt	gccgtctgaa	1740
tttgacctga	gcgcattttt	acgcgcggga	gaaaaccgcc	tcgcggtgat	ggtgctgctg	1800
tggagtgcag	gcagttatct	ggaagatcag	gatattgtgg	ggatgagcgg	cattttccgt	1860
gacgtctcgt	tgttgacata	accgactaca	caaatcagcg	atttccatgt	tgccactcgc	1920
tttaatgatg	atthtcagcg	cgctgtactg	gaggctgaag	ttcagatgtg	cggcgagtgt	1980
cgtgactacc	tacgggtaac	agtttcttta	tggcagggtg	aaacgcagggt	cgccagcggc	2040
accgcgcctt	tcggcggtga	aattatcgat	gagcgtgggtg	gttatgccga	tcgcgtcaca	2100
ctacgtctga	acgtcgaaaa	cccgaacttg	tggagcgccg	aaatcccga	tctctatcgt	2160
gcgggtgggtg	aactgcacac	cgccgacggc	acgctgattg	aagcagaagc	ctgcgatgtc	2220
ggtttcgcgc	aggtgcggtg	tgaatgggtg	ctgctgctgc	tgaacggcaa	gccgttgctg	2280
attcgaggcg	ttaaccgtca	cgagcatcat	cctctgcatg	gtcagggtcat	ggatgagcag	2340
acgatgggtg	aggatatcct	gctgatgaag	cagaacaact	ttaacgcctg	gcgctgttctg	2400
cattatccga	accatccgct	gtggtacacg	ctgtgcgacc	gctacggcct	gtatgtgggtg	2460
gatgaagcca	atattgaaac	ccacggcatg	gtgccaatga	atcgtctgac	cgatgatccg	2520
cgctgggtac	cggcgatgag	cgaacgcgta	acgcgaatgg	tgcagcgcgga	tcgtaatcac	2580
ccgagtgtga	tcatctgggtc	gctggggaaat	gaatcaggcc	acggcgctaa	tcacgacgcg	2640
ctgtatcgct	ggatcaaatc	tgtcgatcct	tcccgcccg	tgcagtatga	aggcggcggga	2700
gccgacacca	cggccaccca	tattatttgc	ccgatgtacg	cgcgcgtgga	tgaagaccag	2760
cccttcccg	ctgtgccgaa	atggtccatc	aaaaaatggc	tttcgctacc	tggagagacg	2820
cgcccgtgta	tcctttgcga	atacgcccac	gcgatgggtg	acagtccttg	cggtttcgtc	2880
aaatactggc	aggcgthtctg	tcagtatccc	cgthttacagg	gcggcttctg	ctgggactgg	2940
gtggatcagt	cgctgattaa	atatgatgaa	aacggcaacc	cgtggctcggc	ttacggcggtg	3000
gattttggcg	atacgccgaa	cgatcgcgag	ttctgtatga	acggctctgg	ctttgcgac	3060
cgcacgcgcg	atccagcgct	gacggaagca	aaacaccagc	agcagthttt	ccagthtccg	3120
ttatccgggg	aaaccatcga	agtgaccagc	gaatacctgt	tccgtcatag	cgataacgag	3180

ctcctgcact	ggatgggtggc	gctgggatggt	aagccgctgg	caagcgggtga	agtgcctctg	3240
gatgtcgctc	cacaaggtaa	acagttgatt	gaactgcctg	aactaccgca	gccggagagc	3300
gccgggcaac	tctggctcac	agtacgcgta	gtgcaaccga	acgcgaccgc	atggtcagaa	3360
gccgggcaca	tcagcgctcg	gcagcagtg	cgtctggcgg	aaaacctcag	tgtgacgctc	3420
cccgcgcgt	cccacgccat	cccgcatctg	accaccagcg	aatggattt	ttgcatcgag	3480
ctgggtaata	agcgttggca	atttaaccgc	cagtcaggct	ttctttcaca	gatgtggatt	3540
ggcgataaaa	aacaactgct	gacgccgctg	cgcgatcagt	tcacccgtgc	accgctggat	3600
aacgacattg	gcgtaagtga	agcgaccgcg	attgacccta	acgcctgggt	cgaacgctgg	3660
aaggcggcgg	gccattacca	ggccgaagca	gcgttggtgc	agtgcacggc	agatacactt	3720
gctgatgcgg	tgctgattac	gaccgctcac	gcgtggcagc	atcaggggaa	aaccttattt	3780
atcagccgga	aaacctaccg	gattgatggt	agtgggtcaa	tggcgattac	cgttgatggt	3840
gaagtggcga	gcgatacacc	gcacccggcg	cggattggcc	tgaactgcca	gctggcgagc	3900
gtagcagagc	gggtaaactg	gctcggatta	ggccgcgaag	aaaactatcc	cgaccgcctt	3960
actgccgcct	gttttgaccg	ctgggatctg	ccattgtcag	acatgtatac	cccgtacgtc	4020
ttcccagagc	aaaacgggtc	gcgctgcggg	acgcgcgaat	tgaattatgg	cccacaccag	4080
tggcgcgggc	acttccagtt	caacatcagc	cgctacagtc	aacagcaact	gatggaaacc	4140
agccatcgcc	atctgctgca	cgcggaagaa	ggcacatggc	tgaatatcga	cggtttccat	4200
atggggattg	gtggcgacga	ctcctggagc	ccgtcagtat	cggcggaatt	tcagctgagc	4260
gccggtcgct	accattacca	gttggctctg	tgtcaaaaat	aataatctcg	aatcaagctt	4320
atcgataccg	tcgaaacttg	tttattgcag	cttataatgg	ttacaaataa	agcaatagca	4380
tcacaaattt	cacaaataaa	gcattttttt	cactgcattc	tagtttgtgt	ttgtccaaac	4440
tcataaatgt	atcttatcat	gtctggatcc	gacctcggat	ctggaagggtg	ctgaggtacg	4500
atgagacccg	caccaggtgc	agaccctgcg	agtgtggcgg	taaacatatt	aggaaccagc	4560
ctgtgatgct	ggatgtgacc	gaggagctga	ggcccgatca	cttgggtgctg	gcctgcaccc	4620
gcgctgagtt	tggctctagc	gatgaagata	cagattgagg	tactgaaatg	tgtgggcgtg	4680
gcttaagggt	gggaaagaat	atataagggt	ggggtcttat	gtagttttgt	atctgttttg	4740
cagcagcgcc	cgccgcctatg	agcaccaact	cgtttgatgg	aagcattgtg	agctcatatt	4800
tgacaacgcg	catgccccca	tgggcccggg	tgcgtcagaa	tgtgatgggc	tccagcattg	4860
atgggtcgcc	cgtcctgccc	gcaaactcta	ctaccttgac	ctacgagacc	gtgtctggaa	4920
cgccgttgga	gactgcagcc	tccgcgcgcg	cttcagccgc	tgcagccacc	gcccgcggga	4980
ttgtgactga	ctttgctttc	ctgagcccg	ttgcaagcag	tgcagcttcc	cgttcatccg	5040
cccgcgatga	caagttgacg	gctctttttg	cacaattgga	ttctttgacc	cgggaaactta	5100
atgtcgtttc	tcagcagctg	ttggatctgc	gccagcaggt	ttctgcccctg	aaggcttcct	5160
cccctcccaa	tgccggtttaa	aacataaata	aaaaaccaga	ctctgttttg	atttggatca	5220
agcaagtgtc	ttgctgtctt	tatttagggg	ttttgcgcgc	gcggtagggc	cgggaccagc	5280
ggctcgcgtc	gttgaggggtc	ctgtgtattt	tttccaggac	gtggtaaagg	tgactctgga	5340
tgttcagata	catgggcata	agcccgctct	tggggtggag	gtagcaccac	tgcagagctt	5400
catgctgcgg	ggtggtgttg	tagatgatcc	agtcgtagca	ggagcgctgg	gcgtggtgcc	5460
taaaaaatgtc	tttcagtagc	aagctgattg	ccaggggcag	gccccttggtg	taagtgttta	5520
caaaagcggt	aagctgggat	gggtgcatac	gtggggatat	gagatgcac	ttggactgta	5580
tttttaggtt	ggctatgttc	ccagccatat	ccctccgggg	attcatgttg	tgcagaacca	5640
ccagcacagt	gtatccggtg	cacttgggaa	atttgtcatg	tagcttagaa	ggaaatgcgt	5700
ggaagaactt	ggagacgccc	ttgtgacctc	caagattttc	catgcattcg	tccataatga	5760
tggcaatggg	cccacgggcg	gcggcctggg	cgaagatatt	tctgggatca	ctaactgtat	5820
agttgtgttc	aggatgagat	cgtcataggc	cattttttaca	aagcgcgggc	ggagggtgcc	5880
agactgcggg	ataatggttc	catccggccc	aggggcgtag	ttacctcac	agatttgcac	5940
ttcccacgct	ttgagttcag	atggggggat	catgtctacc	tgcggggcga	tgaagaaaac	6000
ggtttccggg	gtagggggaga	tcagctggga	agaaagcagg	ttcctgagca	gctgcgactt	6060
accgcagcgg	gtgggcccgt	aaatcacacc	tattaccggg	tgcaactggt	agttaagaga	6120
gctgcagctg	cogtcatccc	tgagcagggg	ggccacttcg	ttaaagcatgt	ccctgactcg	6180
catgttttcc	ctgaccaaata	ccgccagaag	gcgctcgccg	cccagcgata	gcagttcttg	6240
caaggaagca	aagtttttca	acggtttgag	accgtccgcg	gtaggcatgc	ttttgagcgt	6300
ttgaccaagc	agttccaggc	ggtcccacag	ctcggtcacc	tgctctacgg	catctcgatc	6360
cagcataatc	ccctggtttc	cgggttgggg	cggctttcgc	tgtacggcag	tagtcggtgc	6420
tcgtccagac	gggcccagggt	catgtctttc	cacgggcgca	gggtcctcgt	cagcgtagtc	6480
tgggtcacgg	tgaaggggtg	cgctccgggc	tgcgcgctgg	ccaggggtgcg	cttgaggctg	6540
gtcctgctgg	tgctgaagcg	ctgccggctc	tcgccctgcg	cgtcggccag	gtagcattttg	6600
accatgggtg	catagtccag	cccctccgcg	gcgtggccct	tggcgcgcag	cttgcccttg	6660
gaggaggcgc	cgcacggggc	gcagtgcaga	cttttgaggg	cgtagagctt	gggcgcgaga	6720
aataccgatt	ccggggagta	ggcatccgcg	ccgcaggccc	cgcagacggt	ctcgcatctc	6780
acgagccagg	tgagctctgg	ccgttcgggg	tcaaaaacca	ggtttccccc	atgctttttg	6840

-81-

atgcggtttct	tacctctgggt	ttccatgagc	cggtgtccac	gctcgggtgac	gaaaaggctg	6900
tccgtgtccc	ogtatacaga	cttgagaggc	ctgtcctcga	gcggtgttcc	gcggtcctcc	6960
tcgtatagaa	actcggacca	ctctgagaca	aaggctcgcg	tccaggccag	cacgaaggag	7020
gctaagtggg	aggggtagcg	gtcgttgtcc	actaggggtg	ccactcgctc	caggggtgtga	7080
agacacatgt	cgccctcttc	ggcatcaagg	aagggtgattg	gtttgtaggt	gtaggccacg	7140
tgaccgggtg	ttcctgaagg	ggggctataa	aaggggggtg	gggcgcgttc	gtcctcactc	7200
tcttcgcat	cgctgtctgc	gagggccagc	tgttgggggtg	agtactccct	ctgaaaagcg	7260
ggcatgactt	ctgcgctaag	attgtcagtt	tccaaaaacg	aggaggattt	gatattcacc	7320
tggcccgcg	tgatgccttt	gaggggtggc	gcatccatct	ggtcagaaaa	gacaatcttt	7380
ttgttgtcaa	gcttggtggc	aaacgacccg	tagagggcgt	tggacagcaa	cttggcgatg	7440
gagcgcagg	tttggttttt	gtcgcgatcg	gcgcgctcct	tggccgcgat	gttttagctgc	7500
acgtattcgc	cgccaacgca	ccgccattcg	ggaaagacgg	tggtgcgctc	gtcgggcacc	7560
aggtgcacgc	gccaaccgcg	gttgtgcagg	gtgacaagg	caacgctggt	ggctacctct	7620
ccgcgtaggc	gctcgtttgt	ccagcagagg	cgcccgccct	tgccgcgagca	gaatggcggt	7680
aggggggtcta	gctgcgtctc	gtccgggggg	tctgcgtcca	cggtaaagac	cccgggcagc	7740
agggcgcggt	cgaagttagtc	tatcttgcat	ccttgcaagt	ctagcgcctg	ctgccatgcy	7800
cgggcggcaa	gcgcgcgctc	gtatgggttg	agtgggggac	cccattggcat	gggggtgggtg	7860
agcgcggagg	cgtacatgcc	gcaaatgtcg	taaacgtaga	ggggctctct	gagtattcca	7920
agatatgtag	ggtagcatct	tccaccgcgg	atgctggcgc	gcacgtaatc	gtatagtctg	7980
tgccagggag	cgaggagggtc	gggaccgagg	ttgctacggg	cgggctgctc	tgctcggaag	8040
actatctgcc	tgaagatggc	atgtgagttg	gatgatattg	ttggacgctg	gaagacgttg	8100
aagctggcgt	ctgtgagacc	taccgcgtca	gcacacgaag	aggcgtagg	gtcgcgcctg	8160
ttgttgacca	gctcggcggt	gacctgcacg	tctagggcgc	agtagtccag	ggtttctctg	8220
atgatgtcat	acttatcctg	tccctttttt	ttccacagct	cgcggttgag	gacaaactct	8280
tcgcgggtctt	tccagtaactc	ttggatcgga	aaccgctcgg	cctccgaacg	gtaagagcct	8340
agcatgtaga	actggttgac	ggcctggtag	gcgcagcatc	ccttttctac	gggtagcgcg	8400
tatgcctgcy	cggccttcgc	gagcgaggtg	tgggtgagcg	caaagggtgtc	cctgaccatg	8460
actttgaggt	actggtatct	gaagtcagtg	tcgtcgcac	cgccctgctc	ccagagcaaa	8520
aagtcgcgtc	gcttttttga	acgcggattt	ggcagggcga	aggtgacatc	gttgaagagt	8580
atctttcccg	cgcgaggcat	aaagttgcgt	gtgatgcgga	aggggtcccg	cacctcgga	8640
cggttggttaa	ttacctgggc	ggcgagcacg	atctcgtcaa	agccgttgat	gttgtggccc	8700
acaatgtaaa	gttccaagaa	gcgcgggatg	cccttgatgg	aaggcaattt	tttaagttcc	8760
tcgtaggtga	gctcttcagg	ggagctgagc	ccgtgctctg	aaagggccca	gtctgcaaga	8820
tgagggttgg	aagcgacgaa	tgagctccac	aggtcacggg	ccattagcat	ttgcagggtg	8880
tcgcgaaagg	tcctaaactg	gcgacctatg	gccatttttt	ctgggggtgat	gcagtagaag	8940
gtaagcgggt	cttggtccca	gcgggtcccat	cgaaggttcg	cggttaggtc	tcgcgcgcag	9000
gtcactagag	gctcatctcc	gccgaacttc	atgaccagca	tgaagggcac	gagctgcttc	9060
ccaaaggccc	ccatccaagt	ataggtctct	acatcgtagg	tgacaaaagag	acgctcggtg	9120
cgaggatgcy	agccgatcgg	gaagaactgg	atctcccgcc	accaattgga	ggagtggtta	9180
ttgatgtggt	gaaagttaga	gtccctgcga	cgggccgaac	actcgtgctg	gcttttgtta	9240
aaacgtgcgc	agtactggca	gcgggtgcacg	gcctgtacat	cctgcacgag	gttgacctga	9300
cgaccgcgca	caaggaagca	gagtgggaa	ttgagccctt	cgcttgccgg	gtttggctgg	9360
tggtcttcta	cttcggctgc	ttgtccttga	ccgtctgggt	gctcagaggg	agttacgggtg	9420
gatcggacca	ccacgcgcgc	cgagcccaaa	gtccagatgt	ccgcgcgcgc	cggtcggagc	9480
ttgatgacaa	catcgcgcag	atgggagctg	tccatggtct	ggagctccc	cggcgtcagg	9540
tcaggcggga	gctcctgcag	gtttacctcg	catagacggg	tcagggcgcg	ggctagatcc	9600
aggtgatacc	taatttccag	gggtcggttg	gtggcgccgt	cgatggcttg	caagaggccg	9660
catccccgcg	gcgcgactac	ggtagccgcg	ggcgggcggt	gggcgcgcgg	ggtgtccttg	9720
gatgatgc	ctaaaagcgg	tgacgcgggc	gagcccccg	aggtaggggg	ggctccggac	9780
ccgcggggag	agggggcagg	ggcacgctcg	cgccgcgcgc	gggcaggagc	tggtgtctgc	9840
cgcgtaggtt	gctggcgaac	gcgacgacgc	ggcggttgat	ctcctgaatc	tgggcgcctct	9900
gcgtgaagac	gacgggcccc	gtgagcttga	gcctgaaaga	gagttcgaca	gaatcaattt	9960
cggtgtcgtt	gacggcgccc	tggcgcaaaa	tctcctgcac	gtctcctgag	ttgtcttgat	10020
agcgatctc	ggccatgaac	tgctcgatct	cttcctcctg	gagatctccg	cgtccggctc	10080
gctccacggt	ggcggcgagg	tcgttgga	tgccggccat	gagctgcgag	aaggcggtga	10140
ggcctccctc	gttccagacg	cggtgttaga	ccacgcccc	ttcggcacgc	cgggcgcgca	10200
tgaccacctg	cgcgagattg	agctccacgt	gcccggcgaa	gacggcgtag	tttcgcaggc	10260
gctgaaagag	gtagttgagg	gtgggtggcg	ttgtgtctgc	cacgaagaag	tacataaacc	10320
agcgtgcgaa	cgtagattcg	ttgatatccc	ccaaggcctc	aaggcgctcc	atggcctcgt	10380
agaagtccac	ggcgaagttg	aaaaactggg	agttgcgcgc	cgacacggtt	aactcctcct	10440
ccagaagacg	gatgagctcg	gcgacagtgt	cgcgacctc	gcgctcaaag	gctacagggg	10500

cctcttcttc	ttotttcaatc	tcctcttcca	taagggcctc	cccttcttct	tcttctggcg	10560
gcgggtgggg	aggggggaca	cggcggcgac	gacggcgcac	cgggaggcgg	tcgacaaagc	10620
gctcgatcat	ctcccccg	cgacggcgca	tgggtctcgg	gacggcgcg	ccgttctcgc	10680
ggggggcgag	ttggaagacg	ccgcccgtca	tgtcccgggt	atgggttggc	ggggggctgc	10740
catgcgccag	ggatacggcg	ctaacgatgc	atctcaacaa	ttgttgtgta	ggtactccgc	10800
cgcgcaggga	cctgagcgag	tccgcaccca	ccggatcgga	aaacctctcg	agaaaggcgt	10860
ctaaccagtc	acagtcgcaa	ggtaggctga	gcaccgtggc	gggcggcagc	gggcggcggt	10920
cgggggttgt	tctggcgagg	gtgctgctga	tgatgtaatt	aaagtaggcg	gtcttgagac	10980
ggcggttggt	cgacagaagc	accatgtcct	tgggtccggc	ctgctgaatg	cgcaggcggt	11040
cggccatgcc	ccaggcttcg	ttttgacatc	ggcgaggttc	ttttagtag	tcttgcatga	11100
gcctttctac	cggcaactct	tcttctcctt	cctcttgtcc	tgcattctct	gcattctatc	11160
cgaagccctc	catcggtgta	ggcggtaggt	ggcgccctct	tcctcccatg	cgtgtgacct	11220
cctgctgcac	ctgctgagag	agcagggcta	ggtcggcgac	aacgcgctcg	gctaataatg	11280
cgcctgtgtt	gatggtgtaa	gtagactgga	agtcattccat	gtccacaaag	cgggtggtatg	11340
cgggctgcga	gagctcggtg	gtgcagtgtg	ccataacgga	ccagttaacg	gtctggtgac	11400
cgttgcaagt	ccgcaccagg	tacctgagac	gcgagtaagc	cctcgagtca	aatacgtagt	11460
agagggggcca	gcgtagggtg	tactggtatc	ccaccaaaaa	gtgcggcgcc	ggctggcggt	11520
gatatccgta	gatgtacctg	gccggggctc	cggggggcag	atcttccaac	ataaggcgat	11580
gaaagtgcgc	gacgcgggtt	gacatccagg	tgatgccggc	ggcgggtggt	gaggcgcgcg	11640
cgctctgggc	ggtcaggcgc	cagatgttgc	gcagcggcaa	aaagtgtctc	atggctggga	11700
tgtaagcggg	cactcttccg	gcgcaatcgt	tgacgctcta	gaccgtgcaa	aaggagagcc	11760
accgggggtt	gagccccgta	tgtctgtgtg	gataaattcg	caagggtatc	atggcggacg	11820
gtcgaaccca	ggtgtgcgac	tccggccgtc	cgcctgtagc	catgcgggta	ccgcccgcgt	11880
gcgcggcgcc	tgctgcgcta	gtcagacaac	gggggagtg	tccttttggc	ttccttccag	11940
gctggaagc	gaaagcatta	gcttttttgg	ccactggccg	cgcgcagcgt	aagcgggttag	12000
ggtgagtcgc	gggacccccg	agtggctcgc	tcctgttagc	cggagggtta	ttttccaagg	12060
ttgcctcccc	gtcatgcaag	gttcgagctc	cggaccggcc	ggactgcggc	gaacgggggt	12120
ttttttgctt	ttcccagatg	accccgcttg	caaattcctc	cggaaacagg	gacgagcccc	12180
ggcaagagca	agagcagcgg	catccggtgc	tgccgcagat	gcgccccctc	cctcagcagc	12240
gaggggcgac	atccgcgggt	cagacatgca	gggcaccctc	ccctcctcct	accgcgtcag	12300
ggggccggca	ctacctggac	cagatggtga	cagatggtga	ttacgaacct	ccgcggcgcc	12360
ctcctgagcg	gtacccaagg	tgaggaggag	gcgaggccct	ggcgcggtta	ggagcgccct	12420
ggcagaacct	gttttcgagc	gtgcagctga	agcgtgatac	gcgtgaggcg	tacgtgccgc	12480
tccacgcagg	gcgcgagctg	cgcgaggagg	aggagccgga	ggagatgcgg	gatcgaaagt	12540
actttgagcc	cgacgcgcga	cggcatggcc	tgaatcgcca	gcggttgctg	cgcgaggagg	12600
acctggtaac	cgcatacgag	accgggatta	gtcccgcgcg	cgcacacgtg	gcggccgccc	12660
acaaccacgt	gcgtacgctt	cagacgggtga	accaggagat	taactttcaa	aaaagcttta	12720
gggactttgt	aagcgcgctg	gtggcgcgcg	aggaggtggc	tataggactg	atgcatctgt	12780
tcctttatgt	gcagcacagc	gagcaaaacc	caaattagcaa	gcccgtcatg	gcgcagctgt	12840
tagagcccg	gggcgctggg	agggacaacg	agggacttcag	ggatgcgctg	ctaaacatag	12900
aggagcgcag	cctgagcctg	ctgctcgatt	tgataaacat	cctgcagagc	atagtgggtg	12960
tgggcaagtt	ttacgcccgc	gctgacaagg	tggccgccat	caactattcc	atgcttagcc	13020
taaagatcga	ggggttctac	aagatatacc	atacccttta	cgttcccata	gacaaggagg	13080
tgggcgttta	tcgcaacgag	atgcgcattg	cgctgaagg	gcttaccttg	agcgacgacc	13140
tcagcgaccg	cgagctgatg	cgcattccaca	aggcctgag	cgtgagccgg	cgcgcgagc	13200
atagagaggc	cgagtcctac	cacagcctgc	aaaggccctc	ggctggcaag	ggcagcgccg	13260
gcgccttgga	ggcagctggg	tttgacgcgg	gcgctgacct	gcgctggggc	ccaagccgac	13320
acgtcggcgg	cgtggaggaa	gccggacctg	ggctggcggt	ggcaccgcgc	cgcgctggca	13380
actaagcgg	gatgtttctg	tatgacgagg	acgatgagta	cgagccagag	gacggcgagt	13440
ggcgctgcag	agccagccgt	atcagatgat	gcaagacgca	acggacccgg	cgtgctgggt	13500
ccgcacatcg	tcgctgactg	ccggcccttaa	ctccacggac	gactggcgcc	aggctcatgga	13560
ccggctctcc	gcaattctgg	cgcgcaatcc	tgacgcgttc	cggcagcagc	cgcaggccaa	13620
ggtgttgccg	atcgtaaacg	aagcgggtgg	ccggcgcgcg	gcaaaaccca	cgcacgagaa	13680
cctggtctac	gacgcgctgc	cgctggccga	aaacaggggc	atccggcccg	acgagcccg	13740
caacctggac	cggctgggtg	ttcagcgctg	ggctcggtac	aacagcggca	acgtgcagac	13800
gcagcagggc	aacctgggct	gggatgtgcg	cgaggccgtg	gcgcagcggt	agcgcgcgca	13860
caacgtgccc	cggggacagg	ccatgggtgc	actaaacgcc	ttcctgagta	cacagcccgc	13920
gactgagaca	cgcgaaagtg	aggactacac	caacttttgt	agcgcactgc	ggctaattgt	13980
tagacaaggc	ctgcagaccg	aggtgtacca	gtctgggcca	gactattttt	tccagaccag	14040
gggggtgcgg	gctcccacag	taaacctgag	ccaggctttc	aaaaacttgc	aggggctgtg	14100
		gcgaccgcgc	gaccgtgtct	agcttgctga	cgcaccaactc	14160

-83-

gogcctgttg	ctgctgctaa	tagcgccctt	cacggacagt	ggcagcgtgt	cccgggacac	14220
atacctaggt	cacttgctga	cactgtaccg	cgaggccata	ggtcaggcgc	atgtggacga	14280
gcatactttc	caggagatta	caagtgtcag	ccgcgcgtg	gggcaggagg	acacggggcag	14340
cctggaggca	accctaaact	acctgctgac	caaccggcgg	cagaagatcc	cctcgttgca	14400
cagtttaaac	agcgaggagg	agcgcatttt	gcgctacgtg	cagcagagcg	tgagccttaa	14460
cctgatgcgc	gacggggtaa	cgcccagcgt	ggcgctggac	atgaccgcgc	gcaacatgga	14520
accgggcatg	tatgcctcaa	accggccgtt	tatcaaccgc	ctaattggact	acttgcacgc	14580
cgcgcccgcc	gtgaaccccg	agtatttcac	caatgccatc	ttgaacccgc	actggctacc	14640
gccccctggt	ttctacaccg	ggggattcga	ggtgcccag	ggtaacgatg	gattcctctg	14700
ggacgacata	gacgacagcg	tgttttcccc	gcaaccgcag	accctgctag	agttgcaaca	14760
gcgcgagcag	gcagaggcgg	cgctgcgaaa	ggaaagcttc	cgcaggccaa	gcagcttgct	14820
cgatctagcg	gctgcggccc	cgcggtcaga	tgctagtagc	ccattttcaa	gcttgatagg	14880
gtctcttacc	agcactcgca	ccaccgcgcc	gcgctgctg	ggcgaggagg	agtacctaaa	14940
caactcgctg	ctgcagccgc	agcgcgaaaa	aaacctgcct	ccggcatttc	ccaacaacgg	15000
gatagagagc	ctagtggaca	agatgagtag	atggaagacg	tacgcgcagg	agcacaggga	15060
cgtgccaggc	ccgcgcccgc	ccaccgctcg	tcaaaggcac	gaccgtcagc	ggggtctggt	15120
gtgggaggac	gatgactcgg	cagacgacag	cagcgtcctg	gatttgggag	ggagtggcaa	15180
ccggtttgcg	caaccttcgcc	ccaggctggg	gagaatgttt	taaaaaaaaaa	aaagctgat	15240
gcaaaaataaa	aaactcacca	aggccatggc	accgagcgtt	ggttttcttg	tattccccct	15300
agtatgcggc	gcgcggcgat	gtatgaggaa	ggtcctcctc	cctcctacga	gagtgtggtg	15360
agcgcgggcg	cagtggcgcg	ggcgctgggt	tctcccttcg	atgctccctc	ggaccgcgcg	15420
ttttgcoctc	cgcggtacct	cgggcctacc	ggggggagaa	acagcatccg	ttactctgag	15480
ttggcacccc	tattcgacac	caccctgtgt	tacctggtgg	acaacaagtc	aacggatgtg	15540
gcatccctga	actaccagaa	cgaccacagc	aactttctga	ccacggtcat	tcaaaacaat	15600
gactacagcc	cgggggaggc	aagcacacag	accatcaatc	ttgacgaccg	gtcgcactgg	15660
ggcggcgacc	tgaaaaccat	cctgcatacc	aacatgccaa	atgtgaacga	gttcatgttt	15720
accaataagt	ttaaggcgcg	ggtgatgggt	tcgcgtttgc	ctactaagga	caatcagggt	15780
gagctgaaat	acgagtgggt	ggagttcacg	ctgcccagag	gcaactactc	cgagaccatg	15840
accatagacc	ttatgaacaa	cgcgatcgtg	gagcactact	tgaaagtggg	cagacagaa	15900
gggggttctg	aaagcgacat	cggggtaaa	tttgacaccc	gcaacttcag	actgggggtt	15960
gaccccgta	ctggtcctgt	catgcctggg	gtatatataa	acgaagcctt	ccatccagac	16020
atcatttttg	tgccaggatg	cggggtggag	ttcaccacaa	gccgcctgag	caacttgttg	16080
ggcatccgca	agcggaaccc	cttcaggag	ggcttttaga	tcacctacga	tgatctggag	16140
ggtggttaaca	ttcccgcact	gttggatgtg	gacgcctacc	aggcgagctt	gaaagatgac	16200
accgaacagg	gcgggggttg	gcgaggcggc	agcaaacgca	gtggcagcgg	cgcggaagag	16260
aactccaacg	cggcagccgc	ggcaatgcag	ccggtggagg	acatgaacga	tcatgccatt	16320
cgcggcgaca	cctttgccac	acgggctgag	gagaagcgcg	ctgaggccga	agcagcggcc	16380
gaagctgccg	cccccgctgc	gcaacccgag	gtcgagaagc	ctcagaagaa	accggtgatc	16440
aaacccctga	cagaggacag	caagaaacgc	agttacaacc	taataagcaa	tgacagcacc	16500
ttcaccctag	accgcagctg	gtaccttgca	tacaactacg	gcgaccctca	gaccggaatc	16560
cgctcatgga	ccctgctttg	cactcctgac	gtaacctgcg	gctcggagca	ggtctactgg	16620
tcgttgccag	acatgatgca	agaccccgct	accttcgcgt	ccacgcgcca	gatcagcaac	16680
tttccggtgg	tgggcgcgca	gctgttgccc	gtgcactcca	agagcttcta	caacgaccag	16740
gccgtctact	cccaactcat	ccgcccagtt	acctctctga	cccacgtgtt	caatcgcttt	16800
cccgagaacc	agattttggc	gcgcccgcga	gccccaccga	tcaccaccgt	cagtgaatac	16860
gttcctgctc	tcacagatca	cgggacgcta	ccgctgcgca	acagcatccg	aggagtccag	16920
cgagtgaacca	ttactgacgc	cagacgccgc	acctgcccct	acgtttacaa	ggccctgggc	16980
atagctcgcg	cgcgcgtcct	atcgagccgc	acttttttag	caagcatgtc	catccttata	17040
tcgcccagca	ataacacagg	ctggggcctg	cgctttccaa	gcaagatgtt	tggcgggggc	17100
aagaagcgct	ccgaccaaca	ccagtgccgc	gtgcgcgggc	actaccgcgc	gcccctgggg	17160
gcgcacaaac	gcggccgcac	tgggcgcacc	accgtcgatg	acgccatcga	cgcggtgggt	17220
gaggaggcgc	gcaactacac	gccacgcgcg	ccaccagtgt	ccacagtgga	cgcgccatt	17280
cagaccgtgg	tgcgcgggag	ccggcgctat	gctaaaatga	agagacggcg	gaggcgcgta	17340
gcacgtcgcc	accgcgcgcg	accgggcact	gccgcgcaac	gcgcggcggc	ggccctgctt	17400
aaccgcgcac	gtcgcaaccg	ccgacggcg	gccatgcggg	ccgctcgaag	gctggccgcg	17460
ggtattgtca	ctgtgcccc	caggtccagg	cgacgagcgg	ccgcgcgagc	agccgcgggc	17520
attagtgcta	tgactcaggg	tcgcaggggc	aacgtgtatt	gggtgcgcga	ctcggttagc	17580
ggcctgcgcg	tgcgcgtg	cacccgcccc	ccgcgcaact	agattgcaag	aaaaaactac	17640
ttagactcgt	actgttgat	gtatccagcg	cgcgcgccgc	gcaacgaagc	tatgtccaag	17700
cgcaaaatca	aagaagagat	gctccaggct	atcgcgccgg	agatctatgg	ccccccgaag	17760
aaggaagagc	aggattacaa	gccccgaaag	ctaaagcggg	tcaaaaagaa	aaagaaagat	17820

-84-

gatgatgatg	aacttgacga	cgaggtggaa	ctgctgcacg	ctaccgcgcc	caggcgacgg	17880
gtacagtggg	aaggtcgacg	cgtaaaacgt	gttttgcgac	ccggcaccac	cgtagtcttt	17940
acgcccgggtg	agcgctccac	ccgcacctac	aagcgcgtgt	atgatgaggt	gtacggcgac	18000
gaggacctgc	ttgagcaggg	caacgagcgc	ctcggggagt	ttgcctacgg	aaagcggcat	18060
aaggacatgc	tggcgctgcc	gctggacgag	ggcaacccaa	cacctagcct	aaagcccgtg	18120
acactgcagc	aggtgctgcc	cgcgcttgca	ccgtccgaag	aaaagcgcgg	cctaaagcgc	18180
gagtctgggtg	acttggcacc	caccgtgcag	ctgatggtac	ccaagcgcca	gcgactggaa	18240
gatgtcttgg	aaaaaatgac	cgtggaacct	gggctggagc	ccgaggtccg	cgtgcggcca	18300
atcaagcagg	tggcgccggg	actgggcgtg	cagaccgtgg	acgttcagat	acccactacc	18360
agtagcacca	gtattgccac	cgccacagag	ggcatggaga	cacaaacgtc	cccgtttgcc	18420
tcagcgggtg	cggatgccgc	ggtgcaggcg	gtcgtgcggg	ccgcgtccaa	gacctctacg	18480
gaggtgcaaa	cggaccgcgt	gatgtttcgc	gtttcagccc	ccggcgccc	gcgcggttcg	18540
aggaagtacg	gcgcgcgcag	cgcgctactg	ccgaatatg	ccctacatcc	ttccatttgcg	18600
cctacccccg	gctatcgtgg	ctacacctac	cgccccagaa	gacgagcaac	tacccgacgc	18660
cgaaccacca	ctggaacccg	ccgcgcgcgt	cgccgtcgcc	agcccggtgt	ggccccgatt	18720
tccgtgcgca	gggtggctcg	cgaaggaggc	aggaccctgg	tgctgccaac	agcgcgctac	18780
caccccagca	tcgtttaaaa	gccggtcttt	gtggttcttg	cagatatggc	cctcacctgc	18840
cgctccggtt	tcccggtgcc	gggattccga	ggaagaatgc	accgtaggag	gggcatggcc	18900
ggccacggcc	tgacgggcgg	catgctcgt	gcgcaccacc	ggcggcgggc	cgcgctgcac	18960
cgctcgcatg	gcggcggtat	cctgcccctc	cttattccac	tgatcgccgc	ggcgattggc	19020
gccgtgcccc	gaattgcatc	cgtggccttg	caggcgcaga	gacactgatt	aaaaacaagt	19080
tgcatgtgga	aaaaatcaaaa	taaaaagtct	ggactctcac	gctcgcttgg	tcctgttaact	19140
atthttgtaga	atggaagaca	tcaactttgc	gtctctggcc	ccgcgacacg	gctcgcgccc	19200
gttcatggga	aactggcaag	atatcggcac	cagcaatatg	agcgggtggc	ccttcagctg	19260
gggctcgctg	tggagcggca	ttaaaaaattt	cggttccacc	gttaagaact	atggcagcaa	19320
ggcctggaac	agcagcacag	gccagatgct	gagggataag	ttgaaagagc	aaaattttcca	19380
acaaaagggtg	gtagatggcc	tggcctctgg	cattagcggg	gtgggtggacc	tggccaacca	19440
ggcagtgcaa	aataagatta	acagtaagct	tgatccccgc	cctcccgtag	aggagcctcc	19500
accggccggtg	gagacagtg	ctccagaggg	gcgtggcgaa	aagcgtccgc	gccccgacag	19560
ggaagaaaact	ctggtgacgc	aaatagacga	gcctccctcg	tacgaggagg	cactaaagca	19620
aggcctgccc	accacccgtc	ccatcgcgcc	cattgctacc	ggagtgcgtg	gccagcacac	19680
accgtaacg	ctggacctgc	ctccccccgc	cgacacccag	cagaaaacctg	tgctgccagg	19740
cccgcaccgc	gttgttgtaa	cccgtcctag	ccgcgcgtcc	ctgcgcgcgc	ccgccagcgg	19800
tccgcgatcg	ttgcggcccg	tagccagtgg	caactggcaa	agcacactga	acagcatcgt	19860
gggtctgggg	gtgcaatccc	tgaagcgccg	acgatgcttc	tgaatagcta	acgtgtcgta	19920
tgtgtgtcat	gtatcgctcc	atgtcgccgc	cagaggagct	gctgagccgc	cgcgcgcccg	19980
ctttccaaga	tggctacccc	ttcgatgatg	ccgcagtggg	cttacatgca	catctcgggc	20040
caggacgcct	cggagtacct	gagccccggg	ctggtgcagt	ttgcccgcgc	caccgagacg	20100
tacttcagcc	tgaataacaa	gtttagaaac	cccacggttg	cgcttacgca	cgacgtgacc	20160
acagacgggt	cccagcggtt	gacgctgcgg	ttcatcctg	tggaccgtga	ggatactgct	20220
tactcgtaca	agcgcggtt	caccctagct	gtgggtgata	accgtgtgct	ggacatggct	20280
tccacgtact	ttgacatccg	cggcgtgctg	gacaggggccc	ctacttttaa	gccctactct	20340
ggcactgcct	acaacgccct	ggctcccaag	gggtgccccaa	atccttgcca	atgggatgaa	20400
gctgctactg	ctcttgaaat	aaacctagaa	gaagaggacg	atgacaacga	agacgaagta	20460
gacgagcaag	ctgagcagca	aaaaactcac	gtatttgggc	aggcgcccta	ttctggtata	20520
aatattacaa	aggagggtat	tcaaataagg	gtcgaaggct	aaacacctaa	atatgccgat	20580
aaaacatttc	aacctgaacc	tcaaataagg	gaatctcagt	ggtacgaaac	tgaaattaat	20640
catgcagctg	ggagagtcct	taaaaagact	accccaatga	aaccatgtta	cggttcatat	20700
gcaaaaccca	caaatgaaaa	tggagggcaa	ggcattcttg	taaagcaaca	aaatggaaag	20760
ctagaaagtc	aagtggaaat	gcaatttttc	tcaactactg	aggcgaccgc	aggcaatggg	20820
gataaacttga	ctcctaaggt	ggtattgtac	agtgaagatg	tagatataga	aaccccagac	20880
actcatattt	cttacatgcc	cactattaag	gaaggtaact	cacgagaact	aatggggcaa	20940
caatctatgc	ccaacaggcc	taattacatt	gcttttaggg	acaattttat	tggtcctaag	21000
tattacaaca	gcacgggtaa	tatgggtggt	ctggcgggcc	aagcatcgca	gttgaatgct	21060
gttgtagatt	tgcaagcag	aaacacagag	ctttcatacc	agcttttgct	tgattccatt	21120
ggtgatagaa	ccagggtact	ttctatgtgg	aatcaggctg	ttgacagcta	tgatccagat	21180
gttagaatta	ttgaaaatca	tggaaactgaa	gatgaacttc	caaattactg	ctttccactg	21240
ggaggtgtga	ttaatacaga	gactcttacc	aaggtaaaac	ctaaaacagg	tcaggaaaaa	21300
ggatgggaaa	aagatgctac	agaattttca	gataaaaatg	aaataagagt	tggaaataat	21360
tttgccatgg	aaatcaatct	aaatgccaac	ctgtggagaa	atthctctgta	ctccaacata	21420
gcgctgtatt	tgcccagacaa	gctaaagtac	agtccttcca	acgtaaaaat	ttctgataac	21480

-85-

ccaaacacct	acgactacat	gaacaagcga	gtgggtggctc	ccgggttagt	ggactgctac	21540
attaaccttg	gagcacgctg	gtcccttgac	tatatggaca	acgtcaaccc	atttaaccac	21600
caccgcaatg	ctggcctgcg	ctaccgctca	atggttctgg	gcaatggctg	ctatgtgccc	21660
ttccacatcc	aggtgcctca	gaagttcttt	gccattaaaa	acctccttct	cctgccgggc	21720
tcatcacct	acgagtggaa	cttcaggaag	gatgttaaca	tggttctgca	gagctcccta	21780
ggaaatgacc	taaggggtga	cggagccagc	attaagtttg	atagcatttg	cctttacgcc	21840
accttcttcc	ccatggccca	caacaccgcc	tccacgcttg	aggccatgct	tagaaaacgac	21900
accaacgacc	agtcctttta	cgactatctc	tccgccgcca	acatgctcta	ccctataccc	21960
gccaacgcta	ccaacgtgcc	catatccatc	ccctcccgca	actggggcggc	tttccgcgcc	22020
tgggccttca	cgcgccttaa	gactaaggaa	accccatcac	tgggctcggg	ctacgaccct	22080
tattacacct	actctggctc	tataccctac	ctagatggaa	cctttttacct	caaccacacc	22140
tttaagaagg	tggcattac	ctttgactct	tctgtcagct	ggcctggcaa	tgaccgcctg	22200
cttaccctca	cagagtttga	aattaagcgc	tcagttgacg	gggaggggta	caacgttgcc	22260
cagtgttaaca	tgaccaaaga	ctggttcctg	gtacaaatgc	tagctaacta	caacattggc	22320
taccagggct	tctatatccc	agagagctac	aaggaccgca	tgtactcctt	ccttagaaac	22380
ttccagccca	tgagccgtca	ggtgggtggg	gatactaaat	acaaggacta	ccaacaggtg	22440
ggcatcctac	accaacacaa	caactctgga	tttgtgggt	accttgcccc	caccatgcgc	22500
gaaggacagg	cctaccctgc	taacttcccc	tatccgctta	taggcaagac	cgcagttgac	22560
agcattaccc	agaaaaagtt	tctttgcat	cgcacccttt	ggcgcacccc	attctccagt	22620
aactttatgt	ccatggggcgc	actcacagac	ctggggccaaa	accttctcta	cgccaaactcc	22680
gcccacgcgc	tagacatgac	ttttgaggtg	gatcccatgg	acgagccccc	ccttctttat	22740
gttttgggtg	aagtctttga	cgtggtccgt	ctgcaccggc	cgcaccgcgg	cgtcactcaa	22800
accgtgtacc	tgcgcacgcc	cttctcggcc	ggcaacgcca	caacataaag	aagcaagcaa	22860
catcaacaac	agctgccgcc	atgggctcca	gtgagcagga	actgaaagcc	attgtcaaag	22920
atcttggttg	tgggcatat	tttttgggca	cctatgacaa	gcgctttcca	ggctttgttt	22980
ctccacacaa	gctcgcctgc	gccatagtca	atacggccgg	tgcgcagact	gggggcgtac	23040
actggatggc	ctttgcctgg	aaccgcact	caaaaacatg	ctacctcttt	gagccctttg	23100
gcttttctga	ccagcgactc	aagcaggttt	accagtttga	gtacgagtca	ctcctgcgcc	23160
gtagcgccat	tgtctcttcc	cccagccgct	gtataacgct	ggaaaagtcc	acccaaagcg	23220
tacagggggc	caactcggcc	gcctgtggag	tattctgctg	catgtttctc	cacgcctttg	23280
ccaactggcc	ccaaactccc	atggatcaca	accccaccat	gaaccttatt	accgggggtac	23340
ccaactccat	gctcaacagt	cccaggttac	agcccacct	gcgtcgcaac	caggaacagc	23400
tctacagctt	cctggagcgc	cactcgcctt	acttcgcgag	ccacagtgcg	cagattagga	23460
gcgccacttc	tttttgtcac	ttgaaaaaca	tgtaaaaaata	atgtactaga	gacactttca	23520
ataaaggcaa	atgcttttat	ttgtacactc	tcgggtgatt	atttaccctc	acccttgccg	23580
tctgcgcgtg	ttaaaaatca	aagggtttct	gcgcgcacac	gctatgcgcc	actggcaggg	23640
acacgttgcg	atactggtgt	ttagtgtctc	acttaaaactc	aggcacaacc	atccgcggca	23700
gctcggtgaa	gttttctactc	cacaggctgc	gcaccatcac	caacgcgttt	agcaggtcgg	23760
gcgccgatat	cctgaagtgc	cagttggggc	ctccgccttg	cgcgcgcgag	ttgcgataca	23820
cagggttgca	gcactggaac	actatcagcg	ccgggtggtg	cacgctggcc	agcacgctct	23880
tgtcggagat	cagatccgcg	tccaggtcct	ccgcgttgct	cagggcgaa	ggagtcaact	23940
ttggtagctg	ccttcccaaa	aagggcgcgt	gccaggctt	tgagttgcac	tcgcaccgta	24000
gtggcatcaa	aaggtgaccg	tgcccgtctt	ggcggttagg	atacagcgcc	tgcataaaag	24060
ccttgatctg	cttaaaagcc	acctgagcct	ttgcgccttc	agagaagaac	atgccgcaag	24120
acttgccgga	aaactgattg	gcccagacgg	ccgcgtcgtg	cacgcagcac	cttgcgctcg	24180
tgttggagat	ctgcaccaca	tttcggcccc	accggttctt	cacgatcttg	gccttgctag	24240
actgctcctt	cagcgcgcgc	tgcccgtttt	cgctcgtcac	atccatttca	atcacgtgct	24300
ccttatattat	cataatgctt	ccgtgtagac	acttaagctc	gccttcgata	tcagcgcagc	24360
ggtgcagcca	caacgcgcag	cccgtgggct	cgtgatgctt	gtaggteacc	tctgcaaacg	24420
actgcagcta	cgccctgcagg	aatcgcccca	cttcgctcac	aaaggctctg	ttgctgggtga	24480
aggtcagctg	caaccgcggg	tgctcctcgt	tcagccaggt	cctgcatacg	gcccgcagag	24540
cttccacttg	gtcaggcagt	agtttgaaat	tcgccttttag	atcgttatcc	acgtgggtact	24600
tgtccatcag	cgcgcgcgca	gcctccatgc	ccttctccca	cgcagacacg	atcggcacac	24660
tcagcgggtt	catcaccgta	atttcaactt	ccgcttcgct	gggctcttcc	tcttctctct	24720
gcgtccgcat	actcagcgcc	actgggtcgt	cttcattcag	ccgccgcact	gtgcgcttac	24780
ctcctttgcc	atgcttgatt	agcaccgggtg	ggttgctgaa	accaccatt	tgtagcgcca	24840
catcttctct	ttcttctctg	ctgtccacga	ttacctctgg	tgatggcggg	cgtcgggct	24900
tgggagaagg	gcgcttcttt	ttcttctttg	gcgcaatggc	caaatccgcc	gcccaggtcg	24960
atggccgcgg	gctgggtgag	cgcggcacc	tgcgctcttg	tgatgagtct	tctcgtcct	25020
cggactcgat	acgcgcctc	atccgctttt	ttggggcgcc	ccggggaggc	ggcggcgagc	25080
gggacgggga	cgacacgtcc	tccatgggtg	ggggacgtcg	cgcgcgaccg	cgtccgcgct	25140

-86-

cggggggtggt	ttcgcgctgc	tccctcttccc	gactggccat	ttcctttctcc	tataggcaga	25200
aaaagatcat	ggagtcagtc	gagaagaagg	acagcctaac	cgccccctct	gagttcgcca	25260
ccaccgcctc	caccgatgcc	gccaacgcgc	ctaccacctt	ccccgtcgag	gcacccccgc	25320
ttgaggagga	ggaagtgatt	atcgagcagg	acccagggtt	tgtaagcgaa	gacgacgagg	25380
accgctcagt	accaacagag	gataaaaagc	aagaccagga	caacgcagag	gcaaacgagg	25440
aacaagtcgg	gcgggggggac	gaaaggcatg	gcgactacct	agatgtggga	gacgacgtgc	25500
tgttgaagca	tctgcagcgc	cagtgcgcca	ttatctgcga	cgcgttgcaa	gagcgcagcg	25560
atgtgcccc	cgccatagcg	gatgtcagcc	ttgcctacga	acgccaccta	ttctcaccgc	25620
gcgtaccccc	caaacgccaa	gaaaacggca	catgcgagcc	caacccgcgc	ctcaacttct	25680
accccgctatt	tgcggtgcca	gaggtgcttg	ccacctatca	catctttttc	caaaactgca	25740
agataccccc	atcctgccc	gccaaaccgca	gccgagcgga	caagcagctg	gccttgccgc	25800
agggcgctgt	catacctgat	atcgctcgc	tcaacgaagt	gccaaaaatc	tttgagggtc	25860
ttggacgcga	cgagaagcgc	gcggcaaacg	ctctgcaaca	ggaaaacagc	gaaaaacgaa	25920
gtcactctgg	agtgttggtg	gaactcgagg	gtgacaacgc	gcgcctagcc	gtactaaaac	25980
gcagcatcga	ggtcaccac	tttgcctacc	cggcacttaa	cctaccccc	aagggtcatga	26040
gcacagtcac	gagtgagctg	atcgctgcgc	gtgcgcagcc	cctggagagg	gatgcaaatt	26100
tgcaagaaca	aacagaggag	ggcctaccgc	cagttggcga	cgagcagcta	gcgcgctggc	26160
ttcaaacgcg	cgagcctgcc	gacttgagg	agcgacgcaa	actaatgatg	gccgcagtg	26220
tcgttaccgt	ggagcttgag	tgcattgcagc	ggttctttgc	tgacccggag	atgcagcgca	26280
agctagagga	aacattgcac	tacacctttc	gacagggtta	cgtacgccag	gcctgcaaga	26340
tctccaacgt	ggagctctgc	aacctggtct	cctaccttgg	aattttgcac	gaaaaccgcc	26400
ttggggcaaaa	cgtgcttcat	tccacgctca	agggcgaggc	gcgcgcgcgc	tacgtccgtg	26460
actgcgttta	cttatttcta	tgctacacct	ggcagacggc	catgggcgtt	tggcagcagt	26520
gcttgaggga	gtgcaacctc	aaggagctgc	agaaactgct	aaagcaaaac	ttgaaggacc	26580
tatggacggc	cttcaacgag	cgctccgtgg	ccgcgcacct	ggcggacatc	attttccccg	26640
aacgcctgct	taaaaccctg	caacagggtc	tgccagactt	caccagtcaa	agcatgttgc	26700
agaactttag	gaactttatc	ctagagcgct	caggaatctt	gcccgcacc	tgctgtgcac	26760
ttcctagcga	ctttgtgccc	attaagtacc	gcgaatgccc	tccgcccgtt	tggggccact	26820
gctaccttct	gcagctagcc	aactaccttg	cctaccactc	tgacataatg	gaagacgtga	26880
gcggtgacgg	tctactggag	tgctactgtc	gctgcaacct	atgcaccccc	caccgctccc	26940
tggtttgcaa	ttcgcagctg	cttaacgaaa	gtcaaattat	cggtaacctt	gagctgcagg	27000
gtccctcgcc	tgacgaaaa	tccgcggctc	cggggttgaa	actcactccg	gggctgtgga	27060
cgctcggtta	ccttcgcaaa	tttgtacctg	aggactacca	cgcccacgag	attaggttct	27120
acgaagacca	atcccgcgcc	ccaaatgcgg	agcttaccgc	ctgcgtcatt	acccagggcc	27180
acattcttgg	ccaattgcaa	gccatcaaca	aagcccgcca	agagtttctg	ctacgaaagg	27240
gacggggggg	ttacttggac	ccccagtcgc	cgccaggagc	caacccaatc	ccccgcgcg	27300
cgagcccta	tcagcagcag	ccgcggggcc	ttgcttccca	ggatggcacc	caaaaagaag	27360
ctgcagctgc	cgccgccacc	cacggacgag	gaggaatact	gggacagtca	ggcagaggag	27420
gttttggacg	aggaggagga	ggacatgatg	gaagactggg	agagcctaga	cgaggaagct	27480
tccgaggtcg	aagaggtgtc	agacgaaaca	ccgtcacctc	cgttcgcatt	cccctcgccg	27540
gcgcccaga	aatcggcaac	cggttccagc	atggctacaa	cctccgctcc	tcaggcgccg	27600
ccggcactgc	ccgttcgccg	acccaaccgt	agatgggaca	ccactggaac	cagggcgggt	27660
aagtccaagc	agccgccgcc	gttagcccaa	gagcaacaac	agcgccaagg	ctaccgctca	27720
tggcgccggc	acaagaacgc	catagttgct	tgcttgcaag	actgtggggg	caacatctcc	27780
ttcgcccgcc	gctttcttct	ctaccatcac	ggcgtggcct	tccccgtaa	catcctgcat	27840
tactaccgtc	atctctacag	cccatactgc	acggcgggca	gcggcagcgg	cagcaacagc	27900
agcggccaca	cagaagcaaa	ggcgaccgga	tagcaagact	ctgacaaaagc	ccaagaaatc	27960
cacagcggcg	gcagcagcag	gaggaggagc	gctgcgtctg	gcgcccacgc	aaccgctatc	28020
gacccgcgag	cttagaaaaca	ggatttttcc	cactctgtat	gctatatctc	aacagagcag	28080
gggccaagaa	caagagctga	aaataaaaaa	caggtctctg	cgatccctca	cccgcagctg	28140
cctgtatcac	aaaagcgaag	atcagcttcg	gcgcacgctg	gaagacgcgg	aggctctctt	28200
cagtaaatac	tgcgcgctga	ctcttaagga	ctagtttcgc	gccctttctc	aaatttaagc	28260
gcgaaaacta	cgatcatctc	agcggccaca	cccgccgcca	gcacctgtcg	tcagcgccat	28320
tatgagcaag	gaaattccca	cgcctctac	gtggagttac	cagccacaaa	tgggacttgc	28380
ggctggagct	actcaaccgc	tccgcgcccc	ccgaaaccga	atgagcgcgg	gacccacat	28440
gatatcccg	gtcaacggaa	ataaccttaa	tccccgtagt	attctcttgg	aacaggcgcc	28500
tattaccacc	cccgtcccca	ccactgtgg	acttcccaga	tggcccgctg	ccctgggtga	28560
ccaggaaagt	tcaggggcgc	agcttgccgg	cggcttctgc	gacgcccagg	ccgaagttca	28620
gatgactaac	actcagctga	gcgaggtatt	gcgaggtatt	cacagggtgc	ggtcgccccg	28680
gcagggtata	cttggtctcc	gtccggacgg	gacattttcag	cagctcaacg	acgagtcgg	28740
gagctcctcg				atcgccggcg	ccggccgctc	28800

ttcattcaag	cctcgtcagg	caatcctaac	tctgcagacc	tcgtcctctg	agccgcgctc	28860
tggaggcatt	ggaactctgc	aatttattga	ggagtttgtg	ccatcgggtc	actttaaccc	28920
cttctcggga	actcccggcc	actatccgga	tcaatttatt	cctaactttg	acgcggtaaa	28980
ggactcggcg	gacggctacg	actgaatggt	aagtggagag	gcagagcaac	tgcgcctgaa	29040
acacctgggtc	cactgtcgcc	gccacaagtg	ctttgcccg	gactccgggtg	agtttttgcta	29100
ctttgaattg	cccagggatc	atatcgaggg	cccgccgcac	ggcgccgggc	ttaccgcca	29160
gggagagctt	gcccgtagcc	tgattcggga	gtttaccag	cgcctcctgc	tagttgagcg	29220
ggacagggga	ccctgtgttc	tcactgtgat	ttgcaactgt	cctaaccctg	gattacatca	29280
agatctttgt	tgccatctct	gtgctgagta	taataaatac	agaaattaaa	atatactggg	29340
gctcctatcg	ccatcctgta	aacgccaccg	tcttcaccgg	cccaagcaaa	ccaaggcgaa	29400
ccttacctgg	tacttttaac	atctctccct	ctgtgattta	caacagtttc	aaccagacg	29460
gagtgaagt	acgagagaac	ctctccgagc	tcagctactc	catcagaaaa	aacaccaccc	29520
tccttacctg	ccgggaacgt	acgagtcggt	caccggccgc	tgcaaccacac	ctaccgctg	29580
accgtaaac	agactttttc	cggacagacc	tcaataactc	tgtttaccag	aacaggaggt	29640
gagcttagaa	aacccttagg	gtattaggcc	aaaggcgag	ctactgtggg	gtttatgaac	29700
aattcaagca	actctacggg	ctattcta	tcagggttct	ctagaaatgg	acggaattat	29760
tacagagcag	cgctgtctag	aaagacgcag	ggcagcgcc	gagcaacagc	gcatgaatca	29820
agagctccaa	gacatggtta	acttgcacca	gtgcaaaagg	ggtatctttt	gtctgggtaaa	29880
gcaggccaaa	gtcacctacg	acagtaatac	caccggacac	cgcttagct	acaagttgcc	29940
aaccaagcgt	cagaaattgg	tggtcatggt	gggagaaaag	cccattacca	taactcagca	30000
ctcggtagaa	accgaaggct	gcattcactc	acctgtgcaa	ggacctgagg	atctctgcac	30060
ccttattaag	acccgtgtcg	gtctcaaaaga	tcttattccc	tttaactaat	aaaaaaaaat	30120
aataaagcat	cacttactta	aaatcagtta	gcaaatttct	gtccagttta	ttcagcagca	30180
cctccttgcc	ctcctcccag	ctctgggtatt	gcagcttcc	cctggctgca	aactttctcc	30240
acaatctaaa	tggaaatgtca	gtttcctcct	gttcctgtcc	atccgcaccc	actatcttca	30300
tgttgttgca	gatgaagcgc	gcaagaccgt	ctgaagatac	cttcaacccc	gtgatccat	30360
atgacacgga	aaccggctcc	ccaactgtgc	cttttcttac	tcctcccttt	gtatcccca	30420
atgggtttca	agagagtcct	cctgggttac	tctctttg	cctatccgaa	cctctagtta	30480
cctccaatgg	catgcttgcg	ctcaaaatgg	gcaacggcct	ctctctggac	gaggccggca	30540
accttacctc	ccaaaatgta	accactgtga	gccacctct	caaaaaaac	aagtcaaaca	30600
taaaactgga	aatatctgca	cccctcacag	ttacctaga	agccctaact	gtggctgccc	30660
ccgcacctct	aattggtcgcg	ggcaacacac	tcaccatgca	atcacaggcc	ccgctaaccg	30720
tgacgactc	caaacttagc	attgccaccc	aaggacccct	cacagtgtca	gaaggaaagc	30780
tagccctgca	aacatcaggg	cccctcacca	ccaccgatag	cagtaacctt	actatcactg	30840
cctcaccccc	tctaaactact	gccactggta	gcttgggcat	tgacttgaaa	gagccatttt	30900
atacacaaaa	tggaaaacta	ggactaaagt	acggggctcc	tttgcatgta	acagacgacc	30960
taaaactttt	gaccgtagca	actggtccag	gtgtgactat	taataataact	tccttgcaaa	31020
ctaaagttac	tggagccttg	ggttttgatt	cacaaggcaa	tatgcaactt	aatgtagcag	31080
gaggactaag	gattgattct	caaaacagag	gccttatact	tgatgttagt	tatccgtttg	31140
atgctcaaaa	ccaactaaat	ctaagactag	gacagggccc	tctttttata	aactcagccc	31200
acaacttgga	tattaactac	aacaaaggcc	tttacttggt	tacagcttca	aacaattcca	31260
aaaagcttga	ggttaacctc	agcactgcca	aggggttgat	gtttgacgct	acagccatag	31320
ccattaatgc	aggagatggg	cttgaatttg	gttcacctaa	tgccaccaac	acaaatcccc	31380
tcaaaacaaa	aattggccat	ggcctagaat	ttgattcaaa	caaggctatg	gttcctaaac	31440
taggaactgg	ccttagtttt	gacagcacag	gtgccattac	agtaggaaac	aaaaataatg	31500
ataagctaac	tttgtggacc	acaccagctc	catctcctaa	ctgtagacta	aatgcagaga	31560
aagatgctaa	actcactttg	gtcttaacaa	aatgtggcag	tcaaataactt	gctacagttt	31620
cagttttggc	tgttaaaggc	agttttggctc	caatatctgg	aacagttcaa	agtgtcatc	31680
ttattataag	atttgacgaa	aatggagtgc	tactaaacaa	ttccttctctg	gaccagaat	31740
attggaactt	tagaaatgga	gatcttactg	aaggcacagc	ctatacaaac	gctgtggat	31800
ttatgcctaa	cctatcagct	tatccaaaat	ctcacggtaa	aactgccaaa	agtaacattg	31860
tcagtcaagt	ttacttaaac	ggagacaaaa	ctaaacctgt	aacactaac	attacactaa	31920
acggtacaca	ggaaacagga	gacacaactc	caagtgcata	ctctatgtca	ttttcatggg	31980
actggtctgg	ccacaactac	attaatgaaa	tatttggccc	atcctcttac	actttttcat	32040
acattgccc	agaataaaga	atcggttggtg	ttatgtttca	acgtgtttat	ttttcaattg	32100
cagaaaattt	caagtcat	ttcattcagt	agtatagccc	caccaccaca	tagcttatac	32160
agatcacctg	accttaatca	aactcacaga	accctagtat	tcaacctgcc	acctccctcc	32220
caacacacag	agtacacagt	cctttctccc	cggctggcct	taaaaagcat	catatcatgg	32280
gtaacagaca	tattcttagg	tggtatat	cacacgggtt	cctgtcgagc	caaacgctca	32340
tcagtgat	taataaaactc	cccgggcagc	tcacttaagt	tcagtgcgt	gtccagctgc	32400
tgagccacag	gctgctgtcc	aacttgcggt	tgcttaacgg	gcggcgaagg	agaagtccac	32460

-88-

```

gcctacatgg gggtagagtc ataatcgtgc atcaggatag ggcggtggtg ctgcagcagc 32520
gcgcgaataa actgctgccg ccgccgctcc gtccctgcagg aatacaacat ggcagtgggtc 32580
tcctcagcga tgattcgcac cgcccgagc ataaggcgcc ttgtcctccg ggcacagcag 32640
cgcaccctga tctcacttaa atcagcacag taactgcagc acagcaccac aatattgttc 32700
aaaatcccac agtgcaaggc gctgtatcca aagctcatgg cggggaccac agaaccacg 32760
tggccatcat accacaagcg caggtagatt aagtggcgac ccctcataaa cactgtggac 32820
ataaacatta cctcttttgg catgttgtaa ttcaccacct ccgggtacca tataaacctc 32880
tgattaaaca tggcgccatc caccaccatc ctaaacagcg tggccaaaac ctgcccgccg 32940
gctatacact gcagggaacc gggactggaa caatgacagt ggagagccca ggactcgtaa 33000
ccatggatca tcatgctcgt catgatatca atgttggcac aacacaggca cactgacata 33060
cacttcccca ggattacaag ctccctccgc gttagaacca tatcccaggg aacaacccat 33120
tcctgaatca gcgtaaatcc cacactgcag ggaagacctc gcacgtaaat cacttgtgc 33180
attgtcaaa gtttacattc gggcagcagc ggatgatcct ccagtatggg agcgcgggtt 33240
tctgtctcaa aaggaggtag acgatcccta ctgtacggag tgcgccgaga caaccgagat 33300
cgtgttgggt gtagtgtcat gccaaatgga acgcccggac tagtcatatt tcctgaagca 33360
aaaccagggt cggcggtgac aaacagatct tccactctct gcgtctccgg tctcgcgcgt 33420
ttgttagtag ttgtagtata tccactctct caaagcatcc aggcgcccc tggcttcggg 33480
ttctatgtaa actccttcat gcgccgctgc cctgataaca tccaccaccg cagaataagc 33540
cacaccacag caacctacac attcgtttctg cgagtcacac acgggaggag cgggaagagc 33600
tggaagaacc atgttttttt ttttattcca aaagattatc caaaacctca aaatgaagat 33660
ctattaagt aacgcgctcc cctccggtgg cgtgggtcaaa ctctacagcc aaagaacaga 33720
taatggcatt tgtaagatgt tgcacaatgg cttccaaaag gcaaacggcc ctcacgtcca 33780
agtggacgta aaggctaaac ccttcagggt gaatctctct tataaacatt ccagcacctt 33840
caaccatgcc caaataattc tcatctcgcc accttctcaa tatatctcta agcaaattccc 33900
gaatattaag tccggccatt gtaaaaaatc gctccagagc gccctccacc ttcagcctca 33960
agcagcgaat catgattgca aaaattcagg ttccctcacag acctgtataa gattcaaaag 34020
cggaacatta acaaaaatac cgcgatcccg taggtccct cgcaggggcca gctgaacata 34080
atcgtgcagg tctgcacgga ccagcgcggc cacttccccg ccaggaaact tgacaaaaga 34140
accacactg attatgacac gcatactcgg agctatgcta accagcgtag ccccgatgta 34200
agctttgttg catgggcggc gatataaaat acatcgtagt catgctcatg cagataaagg 34320
cctcgcgcaa aaaagaaaag gacaccattt ttctctcaaa catgtctgcg ggtttctgca 34380
ccggaaccac cacagaaaaa aaaaaaacat ttaaacatta gaagcctgtc ttacaacagg 34440
taaacacaaa ataaaataac taagacggac tacggccatg ccggcggtgac cgtaaaaaaa 34500
aaaaacaacc cttataagca gcaccaccga cagctcctcg gtcattgtccg gattcataat 34560
ctggtcaccc tgattaaaaa caggttgatt catcggtcag tgctaaaaag cgaccgaaat 34620
gtaagactcg gtaaacacat cgcaggcgta gagacaacat tacagcccc ataggaggta 34680
agcccggggg aatacatacc aaaaacacat aaacacctga aaaacctcc cacagcggca 34740
taacaaaatt aataggagag agaacaacat acagcgcttc cacagcggca gccataacag 34800
aaatagcacc ctcccgtctc gaaaacctat taaaaaaca gtgcagagcg agtatatata ggactaaaaa 34920
tcagccttac cagtaaaaaa aaagggccaa caccagaaaa ccgcacgcga acctacgccc 34980
tcaatcagtc acagtgtaaa cacaacattc ctcaaactgt cacttccgtt tccccacgtt 35040
atgacgtaac ggttaaagtc ccacaaactt taagaaaact acaattccca acacatacaa gttactccg 35100
agaaacgaaa gccaaaaaac ccccgttccc acgcccgcg ccacgtcaca aactccacc 35160
acgtcacttc ccattttaat ccccgttccc taaggtatat tattgatgat g 35211
cctcattatc atattggctt caatccaaaa

```

<210> 52

<211> 33622

<212> DNA

<213> Artificial Sequence

<220>

<223> Plasmid Av3nBg

<400> 52

```

catcatcaat aatatacctt attttggatt gaagccaata tgataatgag ggggtggagt 60
ttgtgacgtg gcgcggggcg tgggaacggg gcgggtgacg tagtagtgtg gcggaagtgt 120
gatgttgcaa gtgtggcgga acacatgtaa gcgacggatg tggcaaaagt gacgtttttg 180
gtgtgcgccg gtgtacacag gaagtgacaa ttttcgcgcg gtttttaggcg gatgtttag 240
taaatttggg cgtaaccgag taagatttgg ccatttttcgc gggaaaactg aataagagga 300

```

agtgaatct	gaataat	gtgttactca	tagcgcgtaa	tatttgtcta	gggcccgcggg	360
gactttgacc	gtttacgtgg	agactcgccc	agggcgcgcc	ccgatgtacg	ggccagatat	420
acgcgtatct	gaggggacta	gggtgtgttt	agggcgaag	cggggcttcg	gttgtagcgc	480
gttaggagtc	ccctcaggat	atagtagttt	cgcttttgca	tagggagggg	gaaatgtagt	540
cttatgcaat	actctttag	tcttgcaaca	tggtaacgat	gagttagcaa	catgccttac	600
aaggagagaa	aaagcaccgt	gcatgccgat	tggtggaagt	aagggtgtac	gatcgtgcct	660
tattaggaag	gcaacagacg	ggtctgacat	ggattggacg	aaccactgaa	ttccgcattg	720
cagagatatt	gtatttaagt	gcctagctcg	atgataaaag	cgccatttga	ccattcacca	780
cattggtgtg	caacctccggc	cctggccact	ctcttcgcga	tcgctgtctg	cgggggccag	840
ctgttgggct	cgcggttgag	gacaaactct	tcgcggtctt	tccagtactc	ttggatcgga	900
aacctgcgg	cctccgaacg	gtactccgcc	gccgagggac	ctgagcgagt	ccgcacgcac	960
cggatcgga	aacctctcga	gaaaggcgtg	taaccagtca	cagtcgctct	agaactagt	1020
gatcccccg	gtgcaggaa	ttcgatctag	atggataaag	gtccaaaaaa	gaagagaaag	1080
gtagaagacc	ccaaggactt	tccttcagaa	ttgctaagtt	ttttgagtga	ttcactggcc	1140
gtcgttttac	aacgtcgtga	ctgggaaaac	cctggcggtta	cccaacttaa	tcgccttgca	1200
gcacatcccc	ctttcgccag	ctggcgtaat	agcgaagagg	cccgcaaccga	tcgcccttcc	1260
caacagttgc	gcagcctgaa	tggcgaatgg	cgctttgcct	ggtttcgggc	accagaagcg	1320
gtgccgga	gctggctgga	gtcgatcttt	ctcgaggccg	atactgtcgt	cgtccctca	1380
aactggcaga	tgcacgggta	cgatgcgcc	atctacacca	acgtaaccta	tcccattacg	1440
gtcaatccgc	cgtttgttcc	cacggagaat	ccgacgggtt	gttactcgct	cacattta	1500
gttgatgaaa	gctggctaca	ggaaggccag	acgcgaatta	tttttgatgg	cgtaactcg	1560
gcgtttcatc	tgtggtgcaa	cgggcgctgg	gtcggttacg	gccaggacag	tcgtttgccg	1620
tctgaatttg	acctgagcgc	atttttacgc	gccggagaaa	accgcctcgc	ggtgatggg	1680
ctgcgttgga	gtgacggcag	ttatctggaa	gatcaggata	tgtggcggat	gagcggcatt	1740
ttccgtgacg	tctcgttgct	gcataaaccg	actacacaaa	tcagcgattt	ccatggtgcc	1800
actcgcttta	atgatgattt	cagccgcgct	gtactggagg	ctgaagttca	gatgtgcggc	1860
gagttgcgtg	actacctacg	ggtaacagtt	tctttatggc	aggggtgaa	gcaggtcgcc	1920
agcggcaccg	cgcttttcgg	cggtgaaatt	atcgatgagc	gtgggtggta	tgccgatcgc	1980
gtcacactac	gtctgaacgt	cgaaaaccgc	aaactgtgga	gcccgcgaa	cccgaatctc	2040
tatcgctgcg	tgggtgaact	gcacaccgcc	gacggcacgc	tgattgaagc	agaagcctgc	2100
gatgtcggtt	tccgcgaggt	gcggttgaa	aatggtctgc	tgctgctgaa	cggcaagccg	2160
ttgctgattt	gaggcgttaa	ccgtcacgag	catcatctc	tgcatggtca	ggtcatggat	2220
gagcagacga	tggtgcagga	tatcctgctg	atgaagcaga	acaactttaa	cgccgtgcgc	2280
tgttcgcatt	atccgaacca	tccgctgtgg	tacacgctgt	gcgaccgcta	cggcctgtat	2340
gtgggtggatg	aagccaatat	tgaaacccac	ggcatgggtc	caatgaatcg	tctgaccgat	2400
gatccgcgct	ggctaccggc	gatgagcgaa	cgcgtaacgc	gaatggtgca	gcgcgatcgt	2460
aatcacccga	gtgtgatcat	ctggtcgctg	gggaatgaat	caggccacgg	cgctaatac	2520
gacgcgctgt	atcgctggat	caaactctgtc	gatccttccc	gcccgggtgca	gtatgaaggc	2580
ggcggagccg	acaccacggc	caccgatatt	atttgcccga	tgtacgcgcg	cgtggatgaa	2640
gaccagccct	tcccggctgt	gcccgaatgg	tccatcaaaa	aatggctttc	gctacctgga	2700
gagacgcgc	cgctgatcct	ttgcgaatac	gcccacgcga	tgggtaacag	tcttggcggt	2760
ttcgctaaat	actggcaggc	gtttcgtcag	tatccccggt	tacagggcgg	cttcgtctgg	2820
gactgggtgg	atcagtcgct	gattaaatat	gatgaaaacg	gcaaccctgt	gtcggcttac	2880
ggcggtgatt	ttggcgatac	gocgaacgat	cgccagttct	gtatgaacgg	tctggtcttt	2940
gccgaccgca	cgccgcaccc	agcgtgacg	gaagcaaaa	accagcagca	gtttttccag	3000
ttccgtttat	ccgggcaaac	catcgaagt	accagcgaat	acctgttccg	tcatagcgat	3060
aacgagctcc	tgcactggat	gggtggcgctg	gatggtaagc	cgctggcaag	cgggtgaagt	3120
cctctggatg	tcgctccaca	aggtaaacag	ttgattgaac	tgccctgaact	accgcagccg	3180
gagagcgccg	ggcaactctg	gctcacagta	cgcgtagtgc	aaccgaacgc	gaccgcattg	3240
tcagaagccg	ggcacatcag	cgcctggcag	cagtggcgct	tggcggaaaa	cctcagtggt	3300
acgcgtcccg	cgccgtccca	cgccatcccg	catctgacca	ccagcgaaat	ggatttttgc	3360
atcgagctgg	gtaataagcg	ttggcaattt	aaccgcaggt	caggctttct	ttcacagatg	3420
tggattggcg	ataaaaaaca	actgctgacg	ccgctgcgcg	atcagttcac	ccgtgcaccg	3480
ctggataacg	acattggcgt	aagtgaagcg	accgcattg	accctaacgc	ctgggtcgaa	3540
cgctggaag	cggcgggcca	ttaccagcc	gaagcagcgt	tggtgcagtg	cacggcgat	3600
acacttgctg	atgcggtgct	gattacgacc	gctcacgcgt	ggcagcatca	ggggaaacc	3660
ttatttatca	gccggaaaac	ctaccggatt	gatggtagtg	gtcaaatggc	gattaccggt	3720
gatgttgaa	tggcgagcga	tacaccgcat	ccggcgcgga	ttggcctgaa	ctgccagctg	3780
gcgcaggtag	cagagcgggt	aaactggctc	ggattagggc	cgcaagaaaa	ctatcccgac	3840
cgccttactg	cgcgctgtt	tgaccgctgg	gatctgccat	tgctcagacat	gtataccccg	3900
tacgtcttcc	cgagcgaaaa	cggctctgcg	tgccggacgc	gcgaattgaa	ttatggccca	3960

-90-

caccagtggc	gcggcgactt	ccagttcaac	atcagccgct	acagtcaaca	gcaactgatg	4020
gaaaccagcc	atcgccatct	gctgcacgcg	gaagaaggca	catggctgaa	tatcgacggg	4080
ttccatatgg	ggattggtag	cgacgactcc	tggagcccg	cagtatcggc	ggaatttcag	4140
ctgagcgccg	gtcgctacca	ttaccagttg	gtctgggtgc	aaaaataata	atctcgaatc	4200
aagcttatcg	ataccgtcga	aacttgttta	ttgcagctta	taatggttac	aaataaagca	4260
atagcatcac	aaatttcaca	aataaagcat	ttttttcact	gcattctagt	tgtgggttgt	4320
ccaaactcat	caatgtatct	tatcatgtct	ggatccgacc	tcggatctgg	aagggtgctga	4380
ggtacgatga	gacccgcacc	aggtgcagac	cctgcgagtg	tggcggtaaa	catattagga	4440
accagcctgt	gatgctggat	gtgaccgagg	agctgaggcc	cgatcacttg	gtgctggcct	4500
gcacccgcgc	tgagtttgcc	tctagcgatg	aagatacaga	ttgaggtact	gaaatgtgtg	4560
ggcgtggctt	aagggtggga	aagaatatat	aagggtgggg	tcttatgtag	ttttgtatct	4620
gttttcgagc	agccgcgcgc	gccatgagca	ccaactcggt	tgatggaagc	attgtgagct	4680
catatttgac	aacgcgcgatg	cccccatggg	ccgggggtgcg	tcagaatgtg	atgggctcca	4740
gcattgatgg	tcgccccgtc	ctgcccgcga	actctactac	cctgacctac	gagaccgtgt	4800
ctggaacgcc	gttggagact	gcagcctccg	ccgcgcgctc	agccgctgca	gccaccgccc	4860
gcgggattgt	gactgacttt	gctttcctga	gcccgcgttc	aagcagtgca	gcttcccgtt	4920
catccgcccc	cgatgacaag	ttgacggctc	ttttggcaca	attggattct	ttgaccgggg	4980
aacttaatgt	cgtttctcag	cagctgttgg	atctgcgcga	gcaggtttct	gccctgaagg	5040
cttcctcccc	tcccaatgcg	gtttaaaaca	taaataaaaa	accagactct	gtttggattt	5100
ggatcaagca	agtgtccttc	tgtctttatt	taggggtttt	gcgcgcgcgg	tagggccggg	5160
accagcggtc	tcggtcgttg	agggctcctgt	gtattttttc	caggacgtgg	taaagggtgac	5220
tctggatggt	cagatacatg	ggcataagcc	cgctctctgg	gtggaggtag	caccactgca	5280
gagcttcatg	ctgcgggggtg	gtgttgtaga	tgatccagtc	gtagcaggag	cgctgggctg	5340
ggtgcctaaa	aatgtccttc	agtagcaagc	tgattgccag	gggcaggccc	ttggtgtaag	5400
tgttttacaaa	gcggttaagc	tgggatgggt	gcatacgtgg	ggatatgaga	tgcatcttgg	5460
actgtatttt	taggttggct	atgttcccag	ccatatccct	ccggggattc	atgttgtgca	5520
gaaccaccag	cacagtgtat	ccggtgcact	tgggaaattt	gtcatgtagc	ttagaaggaa	5580
atgcgtggaa	gaacttggag	acgcccttgt	gacctccaag	attttccatg	cattcgtcca	5640
taatgatggc	aatgggccc	cgggcgccgg	cctgggcgaa	gatatttctg	ggatcactaa	5700
cgtcatagtt	gtgttccagg	atgagatcgt	cataggccat	ttttacaaa	cgccggcgga	5760
gggtgccaga	ctgcggtata	atggttccat	ccggccagg	ggcgtagtta	ccctcacaga	5820
tttgcatctt	ccacgccttg	agttcagatg	gggggatcat	gtctacctgc	ggggcgatga	5880
agaaaacggg	ttccggggta	ggggagatca	gctgggaaga	aagcagggtc	ctgagcagct	5940
gcgacttacc	gcagccgggtg	ggcccgtaaa	tcacacctat	taccggctgc	aactggtagt	6000
taagagagct	gcagctgccg	tcacccctga	gcaggggggc	cacttcgtta	agcatgtccc	6060
tgactcgcat	gttttccctg	accaaaccgc	ccgaaggcgc	ctgcgcgcgc	agcgatgca	6120
gttcttgcga	ggaagcaaag	tttttcaacg	gtttgagacc	gtccgcgcgt	ggcatgcttt	6180
tgagcggttg	accaagcagt	tccaggcggt	cccacagctc	ggtcacctgc	tctacggcat	6240
ctcgatccag	catatctcct	cgtttcgcgg	gttggggcgg	ccttcgctgt	acggcagtag	6300
tcggtgctcg	tccagacggg	ccagggtcat	gtctttccac	gggcgcaggg	tcctcgtcag	6360
cgtagcttgg	gtcacgggtga	aggggtgcgc	tccgggctgc	gcgctggcca	gggtgcgctt	6420
gaggctgggtc	ctgctgggtc	tgaagcgctg	ccggtcttcg	ccctgcgcgt	cgcccgaggta	6480
gcatttgacc	atgggtgcat	agtccagccc	ctccgcggcg	tggcccttgg	cgcgagctt	6540
gcccttggag	gaggcgccgc	acgaggggca	gtgcagactt	ttgagggcgt	agagcttggg	6600
cgcgagaaat	accgattccg	gggagtaggc	atccgcgcgc	caggccccgc	agacggcttc	6660
gcattccacg	agccagggtga	gctctggccg	ttcggggcca	aaaaccagg	ttcccccatg	6720
ctttttgatg	cgtttctttac	ctctgggttt	catgagccgg	tgtccacgct	cggtgacgaa	6780
aaggctgtcc	gtgtccccgt	atacagactt	gagaggccctg	tcctcgagcg	gtgttccgcg	6840
gtcctcctcg	tatagaaact	cggaccactc	tgagacaaa	gctcgcgtcc	aggccagcac	6900
gaaggaggct	aagtgggagg	ggtagcggtc	gttgtccact	agggggtcca	ctcgctccag	6960
ggtgtgaaga	cacatgtcgc	cctcttcggc	atcaaggga	gtgattgggt	tgtagggtga	7020
ggccacgtga	ccgggtgttc	ctgaaggggg	gctataaaa	gggggtgggg	cgcgcttcgtc	7080
ctcactctct	tccgcgcatgc	tgtctgcgag	ggccagctgt	tgggggtgagt	actccctctg	7140
aaaagcgggc	atgacttctg	cgctaagatt	gtcagtttcc	aaaaacgagg	aggatttgat	7200
attcacctgg	cccgcgggtga	tgcttttgag	ggtggccgca	tcacatctgg	cagaaaagac	7260
aatctttttg	ttgtcaagct	tgggtggcaa	cgaccgcgtg	agggcggttg	acagcaactt	7320
ggcgtatggg	cgcagggttt	ggtttttgtc	gcgatccggc	cgctccttgg	ccgcgatgtt	7380
tagctgcacg	tattcgcgcg	caacgcaccg	ccattcggga	aagacgggtg	tgcgctcgtc	7440
gggcaccagg	tgcacgcgcg	aaccgcggtt	gtcaggggtg	acaagggtcaa	cgctgggtggc	7500
tacctctccg	cgttaggcgtc	cgttgggtcca	cgagagcgcg	ccgccttggc	gcgagcagaa	7560
tggcggttag	gggtctagct	gcgtctcgtc	cggggggtct	gcgtccacgg	taaagacccc	7620

-91-

gggcagcagg	cgcgcgctcga	agtagtctat	cttgcatcct	tgcaagtcta	gcgccctgctg	7680
ccatgcgcgg	gcggaagcg	cgcgctcgta	tgggttgagt	gggggacccc	atggcatggg	7740
gtgggtgagc	gcggaggcgt	acatgccgca	aatgtcgtaa	acgtagaggg	gctctctgag	7800
tattccaaga	tatgtagggg	agcatcttcc	accgcggatg	ctggcgcgca	cgtaatcgta	7860
tagttcgtgc	gagggagcga	ggaggtcggg	accgaggttg	ctacgggcgg	gctgctctgc	7920
tcggaagact	atctgcctga	agatggcatg	tgagttggat	gatatggttg	gacgctggaa	7980
gacgttgaag	ctggcgctcg	tgagacctac	cgcgctcacgc	acgaaggagg	cgtaggagtc	8040
gcgcagcttg	ttgaccagct	cggcgggtgac	ctgcacgtct	agggcgagtc	agtcagggtt	8100
ttccttgatg	atgtcatact	tatcctgtcc	cttttttttc	cacagctcgc	ggttgaggac	8160
aaactcttcg	cggtctttcc	agtactcttg	gatcggaac	ccgtcggcct	ccgaacggta	8220
agagcctagc	atgtagaact	ggttgacggc	ctggtagggc	cagcatccct	tttctacggg	8280
tagcgcgat	gcctgcgcgg	ccttcgggag	cgaggtgtgg	gtgagcgcaa	aggtgtccct	8340
gaccatgact	ttgaggtact	ggatattgaa	gtcagtgctg	tcgcatccgc	cctgctccca	8400
gagcaaaaag	tccgtgcgct	ttttggaacg	cggatttggt	agggcgagg	tgacatcggt	8460
gaagagtatc	tttcccgcgc	gaggcataaa	gttgcggtgtg	atgcggaagg	gtcccggcac	8520
ctcggaacgg	ttgttaatta	cctgggcggc	gagcacgac	tcgtcaaaag	cgttgatgtt	8580
gtggcccaca	atgtaaagtt	ccaagaagcg	cgggatgccc	ttgatggaag	gcaatttttt	8640
aagttcctcg	taggtgagct	cttcaggsga	gctgagcccg	tgctctgaaa	gggcccagtc	8700
tgcaagatga	gggttggaag	cgacgaatga	gctccacagg	tcacgggcca	ttagcatttg	8760
caggtggtcg	cgaaagggtcc	taaactggcg	acctatggcc	attttttctg	gggtgatgca	8820
gtagaaggta	agcgggtctt	gttcccagcg	gtcccaccca	aggttcgcgg	ctaggtctctg	8880
cgcggcagtc	actctccgcc	gaacttcacg	accagcatga	accagcatga	agggcacgag	8940
ctgcttccca	aaggccccc	tccaagtata	ggctctctaca	tcgtagggtga	caaagagacg	9000
ctcggtgcga	ggatgcgagc	cgatcgggaa	gaactggatc	tcccgccacc	aatttgaggga	9060
gtggctattg	atgtggtgaa	agtagaagtc	cctgcgacgg	gocgaacact	cggtgtggct	9120
tttgtaaaaa	cgtagcagtc	actggcagcg	gtgcacgggc	tgtacatcct	gcacgaggtt	9180
gacctgacga	ccgcgcacaa	ggaagcagag	tgggaatttg	agccctcgc	ctggcggggt	9240
tggtctggtg	tcttctactt	cggctgcttg	tccttgaccg	tctggctgct	cgaggggagt	9300
tacggtggat	cggaccacca	cgccgcgcga	gccc aaagt	cagatgtccg	cgcgcgcgcg	9360
tcggagcttg	atgacaacat	cgcgagatg	ggagctgtcc	atgggtctgga	gctcccgcgg	9420
cgtcaggtca	ggcggggagct	cctgcaggtt	tacctgcac	agacgggtca	gggcgcgggc	9480
tagatccagg	tgatacctaa	tttccagggt	ctgggttggt	gcggcgtcga	tggcttgcaa	9540
gaggccgcat	ccccgcggcg	cgactacggg	accgcgcggc	gggcgggtgg	ccgcgggggt	9600
gtccttggtg	gatgcaccta	aaagcgggtg	cgcgggcgag	cccccgagg	tagggggggc	9660
tccggaccgc	ccgggagagg	gggcaggggc	acgtcggcgc	cgcgcgcggg	caggagctgg	9720
tgctgcgcgc	gtaggttgct	ggcgaaacgc	agcagcggc	ggttgatctc	ctgaatctga	9780
cgctcttgcg	tgaagacgac	gggcccgggt	agcttgagcc	tgaagagag	ttcgacagag	9840
tcaatttcgg	tgctggtgac	ggcggcctgg	cgcaaaatct	cctgcacgtc	tctgagtttg	9900
tcttgatagg	cgatctcggc	catgaactgc	tcgatctctt	cctcctggag	atctccgcgt	9960
ccggtcgcgt	ccacgggtgc	ggcgaggtcg	ttggaaatgc	gggccatgag	ctgcgagaag	10020
gcgttgaggc	ctccctcggt	ggcgaaacgc	ctgtagacca	cgcccccttc	ggcatcgcg	10080
gcgcgcgatga	ccacctgcgc	gagattgagc	tcacgtgccc	gggcgaagac	ggcgtaggtt	10140
cgcaggcgct	gaaagaggta	gttgagggtg	gtggcggtgt	gttctgccac	gaagaagtac	10200
ataaccacgc	gtcgcaacgt	ggattcggtg	atatccccc	aggcctcaag	gcgctccatg	10260
gcctcgtaga	agtcacacgc	gaagttagaa	aactgggagt	tgccgcgccga	cacgggttaac	10320
tcctctccca	gaagacggat	gagctcggcg	acagtgtcgc	gcacctcgcg	ctcaaaggct	10380
acaggggcct	cttcttcttc	ttcaatctcc	tcttccataa	gggcctcccc	ttcttcttct	10440
tctggcgggc	gtgggggagg	ggggacacgc	cggcgacgac	ggcgaccgg	gaggcggtcg	10500
acaaagcgct	cgatcatctc	cccgcggcga	cggcgcatgg	tctcggtgac	ggcgcgggcg	10560
ttctcgcggg	ggcgaggttg	gaagacgcgc	ccgctcatgt	cccgggttatg	gggtggcggg	10620
gggctgccat	gcggcgaggga	tacggcgcta	acgatgcac	tcaacaattg	ttgtgtaggt	10680
actccgcgc	cgagggacct	gagcgagtc	gcacgacgc	gatcggaaaa	cctctcgaga	10740
aaggcgtcta	accagtcaca	gtcgcaagg	aggctgagca	ccgtggcggg	cggcagcggg	10800
cggcggtcgg	gggtgtttct	ggcgagggtg	ctgctgatga	tgtaattaaa	gtaggcggtc	10860
ttgagacggc	ggatgggtcga	cagaagcaac	atgtccttgg	gtccggcctg	ctgaatgcgc	10920
aggcggtcgg	ccatgcccc	ggcttcggtt	tgacatcggc	gcaggtcttt	gtagtagtct	10980
tgcattgagcc	tttctacggg	cacttcttct	tctccttctc	cttgtcctgc	atctcttgca	11040
tctatcgctg	cggcgggcgg	ggagtttggt	cgtaggtggc	gccctcttcc	tcccatgcgt	11100
gtgaccccca	agccctcat	cggctgaagc	agggctaggt	cggcgacaac	gcgctcgggt	11160
aatatggcct	gctgcacctg	cgtgagggtg	gactggaagt	catccatgtc	cacaaagcgg	11220
tggtagcgcg	ccgtgttgat	ggtgtaagtg	cagttggcca	taacggacca	gttaacgggtc	11280

-92-

tggtgacccg	gctgagagag	ctcggtgtac	ctgagacgcg	agtaagccct	cgagtcaaat	11340
acgtagtcgt	tgcaagtccg	caccaggtac	tggtatccca	ccaaaaagt	cgggcgccgc	11400
tgggcggtaga	ggggccagcg	taggggtggcc	ggggctccgg	gggcgagatc	ttccaacata	11460
aggcgatgat	atccgtagat	gtacctggac	atccaggtga	tgccggcggc	gggtggtggag	11520
gcgcgcggaa	agtcgcggac	gcggttccag	atgttgcgca	gcggcaaaaa	gtgctccatg	11580
gtcgggacgc	tctggccggg	caggcgcgcg	caatcgttga	cgctctagac	cgtgcaaaaag	11640
gagagcctgt	aagcgggcac	tcttccgtgg	tctggtggat	aaattcgcaa	gggtatcatg	11700
gcggacgacc	ggggttcgag	ccccgtatcc	ggcgtccgc	cgatgatccat	gcggttaccg	11760
cccgcggtgc	gaaccaggt	gtgcgacgtc	agacaacggg	ggagtgtctc	ttttggcttc	11820
cttccaggcg	cggcggtgc	tgcgctagct	tttttgccca	ctggccgcgc	gcagcgtaag	11880
cggttaggct	ggaaagcgaa	agcattaagt	ggctcgctcc	ctgtagccgg	agggttattt	11940
tccaagggtt	gagtcgagg	acccccggtt	cgagtcctcg	accggccgga	ctgcggcgaa	12000
cggggggtttg	cctccccgtc	atgcaagacc	ccgcttgcaa	attcctccgg	aaacagggac	12060
gagccctttt	tttgcctttt	ccagatgcat	ccggtgctgc	ggcagatgcg	ccccctcct	12120
cagcagcggc	aagagcaaga	gcagcggcag	acatgcagg	caccctcccc	tctcctacc	12180
gcgtcaggag	gggcgacatc	cgcggttgac	gcggcagcag	atggtgatta	cgaacccccg	12240
cggcgccggg	cccggaacta	cctggacttg	gaggagggcg	agggcctggc	gcggctagga	12300
gcgccctctc	ctgagcggta	cccaagggtg	cagctgaagc	gtgatacgcg	tgaggcgtag	12360
gtgccgcggc	agaacctgtt	tgcgaccgc	gagggagagg	agcccgagga	gatgcgggat	12420
cgaaagtcc	acgcaggcg	cgagctgcgg	catggcctga	atcgcgagcg	gttgctgcgc	12480
gaggaggact	ttgagcccga	cgcgcgaaac	gggattagtc	ccgcgcgcgc	acacgtggcg	12540
gccgccgacc	tggtaacgc	atacgagcag	acggtgaacc	aggagattaa	ctttcaaaaa	12600
agctttaaca	accacgtgcy	tacgcttggt	gcgcgcgagg	agggtggctat	aggactgatg	12660
catctgtggg	actttgttaag	cgcgctggag	caaaacccaa	atagcaagcc	gctcatggcg	12720
cagctgttcc	ttatagtgc	gcacagcagg	gacaacgagg	cattcaggga	tgcgctgcta	12780
aacatagtag	agcccagagg	ccgctggctg	ctcgatttga	taaacaatcct	gcagagcata	12840
gtggtgcagg	agcgcagctt	gagcctggct	gacaagggtg	ccgccatcaa	ctattccatg	12900
cttagcctgg	gcaagtttta	cgcccgcaag	atataccata	ccccttacgt	tcccatagac	12960
aaggaggtaa	agatcgagg	gttctacatg	cgcatggcgc	tgaagggtgt	taccttgagc	13020
gacgacctgg	gcgttttatcg	caacgagcgc	atccacaagg	ccgtgagcgt	gagccggcgg	13080
cgcgagctca	gcgaccgcga	gctgatgcac	agcctgcaaa	gggccctggc	tggcacgggc	13140
agcggcgata	gagaggccga	gtcctacttt	gacgcggggc	ctgacctgcy	ctgggccccca	13200
agccgacgcg	ccctggaggc	agctggggcc	ggacctgggc	tggcggtggc	accgcgcgcg	13260
gctggcaacg	tcggcggcgt	ggaggaatat	gacgaggacg	atgagtacga	gccagaggac	13320
ggcgagtagt	aagcgggtgat	gtttctgac	agatgatgca	agacgcaacg	gaccggcgcg	13380
tgcggggcgg	gctgcagagc	cagccgtccg	gccttaactc	cacggacgac	tggcgccagg	13440
tcattggacc	catcatgtcg	ctgactgcgc	gcaatcctga	cgcgttccgg	cagcagccgc	13500
aggccaaccg	gctctccgca	attctggaag	cggtgggtcc	ggcgcgcgca	aacccccacg	13560
acgagaaggt	gctggcgatc	gtaaacgcgc	tgggcgaaaa	cagggccatc	cggccccgacg	13620
aggccggcct	ggtctacgac	gcgctgcttc	agcgcgtggc	tcgttacaac	agcggcaacg	13680
tgcagaccaa	cctggaccgg	ctggtggggg	atgtgcgcga	ggccgtggcg	cagcgtgagc	13740
gcgcgcagca	gcagggaac	ctgggtccca	tggttgcaact	aaacgccttc	ctgagtacac	13800
agcccgccaa	cgtgccgcgg	ggacaggagg	actacaccaa	ctttgtgagc	gcaactgcggc	13860
taatggtgac	tgagacaccg	caaagtgagg	tgtaccagtc	tgggcccagac	tatttttttcc	13920
agaccagtag	acaaggcctg	cagaccgtaa	acctgagcca	ggctttcaaa	aacttgccagg	13980
ggctgtgggg	ggtgcgggct	cccacaggcg	accgcgcgac	cgtgtctagc	ttgctgacgc	14040
ccaactcgcg	cctggtgctg	ctgctaatag	cgcccttcac	ggacagtggc	agcgtgtccc	14100
gggacacata	cctaggtcac	ttgctgacac	tgtaccgcga	ggccatagg	caggcgcatg	14160
tgagcagagca	tactttccag	gagattacaa	gtgtcagccg	cgcgctgggg	caggaggaca	14220
cgggcagcct	ggaggcaacc	ctaaactaac	tgctgaccaa	ccggcggcag	aagatccctc	14280
cgtttgaacg	tttaaacagc	gaggaggagc	gcatttttgcg	ctacgtgcag	cagagcgtga	14340
gccttaacct	gatgcgcgac	ggggtaacgc	ccagcgtggc	gctggacatg	accgcgcgca	14400
acatggaacc	gggcatgtat	gcctcaaac	ggccgtttat	caaccgccta	atggactact	14460
tgcctgcgcg	ggcgcccggt	aaccccgagt	atttcaccaa	tgccatcttg	aaccgcact	14520
ggctaccgcc	ccctggtttc	tacaccgggg	gattcagagt	gcccaggggt	aacgatggat	14580
tcctctggga	cgacatagac	gacagcgtgt	tttccccgca	accgcagacc	ctgctagagt	14640
tgcaacagcg	cgagcaggca	gaggcggcgc	tgcgaaagga	aagcttccgc	aggccaagca	14700
gcttgctccg	tctaggcgct	gcgccccgcg	ggtcagatgc	tagtagccca	tttccaagct	14760
tgatagggtc	tcttaccagc	actcgcacca	ccgcgccgcg	cctgctgggc	gaggaggagt	14820
acctaataca	ctcgctgctg	cagccgcagc	gcgaaaaaaa	cctgcctccg	gcattttcca	14880
acaacgggat	agagagccta	gtggacaaga	tgagtagatg	gaagacgtac	gcgcaggagc	14940

-93-

acagggacgt	gccaggcccc	cgccccccca	cccgtcgtca	aaggcacgac	cgtcagcggg	15000
gtctgggtgtg	ggaggacgat	gactcggcag	acgacagcag	cgctcctggat	ttgggaggga	15060
gtggcaaaccc	gtttgcgcac	cttcgccccca	ggctgggggag	aatgttttaa	aaaaaaaaaa	15120
gcatgatgca	aaataaaaaa	ctcaccaagg	ccatggcacc	gagcgttggg	tttcttgat	15180
cccccttagt	atgcggcgcg	cggcgatgta	tgaggaaggt	cctcctccct	cctacgagag	15240
tgtggtgagc	gcggcgccag	tggcgggcggc	gctgggttct	cccttcgatg	ctccccctgga	15300
cccgcggtt	gtgcctccgc	ggtacctgcg	gcctaccggg	gggagaaaca	gcatccgtta	15360
ctctgagttg	gcacccctat	tcgacaccac	ccgtgtgtac	ctgggtggaca	acaagtcaac	15420
ggatgtggca	tccctgaact	accagaacga	ccacagcaac	tttctgacca	cggtcattca	15480
aaacaatgac	tacagcccgg	gggaggcaag	cacacagacc	atcaatcttg	acgaccggtc	15540
gcactggggc	ggcgacctga	aaaccatcct	gcataccaac	atgccaaatg	tgaacgagtt	15600
catgtttacc	aataagttta	aggcgcgggg	gatggtgtcg	cgcttgcccta	ctaaggacaa	15660
tcaggtggag	ctgaaatacg	agtgggtgga	ggtggagctg	cccaggggca	actactccga	15720
gaccatgacc	atagacctta	tgaacaacgc	gatcgtggag	cactacttga	aagtgggcag	15780
acagaacggg	gttctggaaa	gcgacatcgg	ggtaaagttt	gacacccgca	acttcagact	15840
gggggttgac	cccgtcactg	gtcttgtcat	gcctggggta	tatacaaacg	aagccttcca	15900
tcagagacac	attttgctgc	caggatgcgg	ggtggacttc	acccacagcc	gcctgagcaa	15960
cttggtgggc	atccgcaagc	ggcaaccctt	ccaggagggc	tttaggatca	cctacgatga	16020
tctggagggt	ggtaacatbc	ccgcactgtt	ggatgtggac	gcctaccagg	cgagcttgaa	16080
agatgacacc	gaacagggcg	gggggtggcg	aggcggcagc	aacagcagtg	gcagcggcgc	16140
ggaagagaac	tccaacgcgg	cagccgcggc	aatgcagccg	gtggaggaca	tgaacgatca	16200
tgccattcgc	ggcgacacct	ttgccacacg	ggctgaggag	aagcgcgctg	aggccgaagc	16260
agcggccgaa	gctgccgccc	ccgctgcgca	acccgagggtc	gagaagcctc	agaagaaacc	16320
ggtgatcaaa	cccctgacag	aggacagcaa	gaaacgcagt	tacaacctaa	taagcaatga	16380
cagcaccttc	acccagtacc	gcagctggta	ccttgcatat	aactacggcg	accctcagac	16440
cggaaacggt	tcctggaccc	tgctttgcac	tcttgacgta	acctgcggct	cggagcaggt	16500
ctactggtcg	ttgccagaca	tgatgcaaga	ccccgtgacc	ttccgctcca	cgcgcagat	16560
cagcaacttt	ccggtgggtg	gcgccgagct	gttgcccgtg	cactccaaga	gcttctacaa	16620
cgaccaggcc	gtctactccc	aactcatccg	ccagtttaac	tctctgaccc	acgtgttcaa	16680
tcgctttccc	gagaaccaga	ttttggcgcg	cccgccagcc	cccaccatca	ccaccgtcag	16740
tgaaaacggt	cctgctctca	cagatcacgg	gacgctaccg	ctgcgcaca	gcatcggagg	16800
agtccagcga	gtgaccatta	ctgacgccag	acgccgcacc	tgccccctacg	tttacaaggc	16860
cctgggcata	gtctcgccgc	gcgtcctatc	gagccgcact	ttttgagcaa	gcatgtccat	16920
ccttatatcg	cccagcaata	acacaggctg	gggcctgcgc	ttcccaagca	agatgtttgg	16980
cgggggccaag	aagcgctccc	accaacaccc	agtgcgcgtg	cgcggggcact	accgcgcgcg	17040
ctggggcgcg	cacaaacgcg	gccgcactgg	gcgcaccaac	gtcgatgacg	ccatcgacgc	17100
ggtggtggag	gaggcgcgca	actacacgcc	cacgcgcgca	ccagtgtcca	cagtggacgc	17160
ggccattcag	accgtgggtgc	gcggagcccc	gcgctatgct	aaaatgaaga	gacggcggag	17220
gcgcgttaac	cgtcgccacc	gccgccgacc	cggcactgcc	gcccacacgcg	cggcgggcggc	17280
cctcgcttaac	gcgcgacgtc	gcaccggccg	acggggcgcc	atgcggggccg	ctcgaaggct	17340
ggcgcggggg	attgtcactg	tgcccccccg	gtccaggcga	cgagcggccg	ccgcgacgc	17400
cgcggccatt	agtgtctatga	ctcagggtcg	caggggcaac	gtgtattggg	tgccgcgactc	17460
ggttagcggc	ctgcgcgtgc	ccgtgcgcac	ccgccccccg	cgcaactaga	ttgcaagaaa	17520
aaactactta	gactcgtact	gttgtatgta	tccagcggcg	gcggcgcgca	acgaagctat	17580
gtccaagcgc	aaaatcaaa	aagagatgct	ccaggtcac	gcgcgggaga	tctatggccc	17640
ccgaagaag	gaagagcagg	attacaagcc	ccgaaagcta	aagcgggtca	aaaagaaaaa	17700
gaaagatgat	gatgatgaac	ttgacgacga	ggtggaactg	ctgcacgcta	ccgcgcccag	17760
gcgacgggta	cagtggaaag	gtcgacgcgt	aaaacgtgtt	ttgcgacccg	gcaccaccgt	17820
agtctttacg	cccgggtgag	gctccaccgc	cacctacaag	cgctgtatg	atgaggtgta	17880
cggcgacgag	gacctgcttg	agcaggccaa	cgagcgcttc	ggggagtttg	cctacggaaa	17940
gcggcataag	gacatgctgg	cgttgccgct	ggacgagggc	aacccaacac	ctagcctaaa	18000
gcccgttaaca	ctgcagcagg	tgctgcccgc	gcttgccaccg	tccgaagaaa	agcgcggcct	18060
aaagcgcgag	tctgggtgact	tggcaccacc	cgtgcagctg	atggtaccca	agcgcacgag	18120
actggaagat	gtcttgga	aaatgacct	ggagctggg	ctggagcccg	aggtcccgct	18180
gcggccaatc	aagcaggtgg	cgccgggact	ggcgctgcag	accgtggacg	ttcagatacc	18240
cactaccagt	agcaccagta	ttgccaccgc	cacagagggc	atggagacac	aaacgtcccc	18300
ggttgccctca	gcggtggcgg	atgcccggt	gcaggcggtc	gctgcggccg	cgtccaagac	18360
ctctacggag	gtgcaaacgg	acccgtggat	gtttcgcgtt	tcagcccccc	ggcgccccgcg	18420
cgttctcgag	aagtagcggc	ccgccagcgc	gttactgccc	gaatatgcc	tacatccttc	18480
cattgcgcct	acccccggct	atcgtggcta	cacctaccgc	cccagaagac	gagcaactac	18540
ccgacgccga	accaccactg	gaaccgcgcg	ccgcgcgtcg	cgtgcgccagc	ccgtgctggc	18600

-94-

cccgattttcc	gtgcgccagg	tggctcgcga	aggaggcagg	accctgggtgc	tgccaacagc	18660
gcgctaccac	cccagcatcg	tttaaaagcc	ggtctttgtg	gttcttgagc	atatggccct	18720
cacctgccgc	cctcgtttcc	cgggtgccggg	attccgagga	agaatgcacc	gtaggagggg	18780
catggccggc	cacggcctga	cgggcggcat	gcgtcgtgcg	caccaccggc	ggcggcgcg	18840
gtcgcaccgt	cgcctgcgcg	gcggtatcct	gcccctcctt	attccactga	tcgcccgggc	18900
gattggcgcc	gtgcccggaa	ttgcatccgt	ggccttgagc	gcgcagagac	actgattaaa	18960
aacaagttgc	atgtggaaaa	atcaaaataa	aaagtctgga	ctctcacgct	cgcttggtcc	19020
tgtaactatt	ttgtagaatg	gaagacatca	actttgcgtc	tctggccccg	cgacacggct	19080
cgcgcccggt	catgggaaac	tggcaagata	tcggcaccag	caatatgagc	ggtggcgcc	19140
tcagctgggg	ctcgctgtgg	agcggcatta	aaaatttcgg	ttccaccggt	aagaactatg	19200
gcagcaaggc	ctggaacagc	agcacaggcc	agatgctgag	ggataagttg	aaagagcaaa	19260
atttccaaca	aaagggtgga	gatggcctgg	cctctggcat	tagcgggggtg	gtggacctgg	19320
ccaaccaggc	agtgcataat	aagattaaca	gtaagcttga	tccccgccct	cccgtagagg	19380
agcctccacc	ggcctgggag	acagtgtctc	cagaggggag	tggcgaaaag	cgctcgcgcc	19440
ccgacaggga	agaaactctg	gtgacgcaaa	tagacgagcc	tccctcgtac	gaggaggcac	19500
taaagcaagg	cctgcccacc	acccgtccca	tcgcgcccac	ggctaccgga	gtgctggggc	19560
agcacacacc	cgtaacgctg	gacctgcttc	ccccgcgca	caccagcag	aaacctgtgc	19620
tgccaggccc	gaccgcccgt	gttgtaaccc	gtcctagccg	cgctccctg	cgccgcgcgc	19680
ccagcgggtc	gcgatcggtg	cggcccgtag	ccagtggcaa	ctggcaaagc	acactgaaca	19740
gcctcgtggg	tctgggggtg	caatccctga	agcgcgcagc	atgcttctga	atagctaacc	19800
tgtcgtatgt	gtgtcatgta	tgcgtccatg	tcgcccgcag	aggagctgct	gagccgcccgc	19860
gcgcccgtct	tccaagatgg	ctacccttcc	gatgatgcgc	cagtgggtctt	acatgcacat	19920
ctcgggcccag	gacgcctcgg	agtacctgag	ccccgggctg	gtgcagtttg	cccgcgccac	19980
cgagacgtac	ttcagcctga	ataacaagtt	tagaaacccc	acggtggcgc	ctacgcacga	20040
cgtgaccaca	gaccgggtccc	agcgtttgac	gctgcgggtc	atccctgtgg	accgtgagga	20100
tactgcgtac	tcgtacaagg	cgcgggtcac	cctagctgtg	ggtgataacc	gtgtgctgga	20160
catggcttcc	acgtactttg	acatccgcgg	cgctgcggac	aggggcccta	cttttaagcc	20220
ctactctggc	actgcctaca	acgcccctgg	tcccaagggt	gccccaaatc	cttgcgaaatg	20280
ggatgaagct	gctactgctc	ttgaaataaa	cctagaagaa	gaggacgatg	acaacgaaga	20340
cgaagtagac	gagcaagctg	agcagcaaaa	aactcacgta	tttgggcagg	cgcccttattc	20400
tggtataaat	attacaaaagg	agggatttca	aatagggtgt	gaagggtcaaa	cacctaaata	20460
tgccgataaa	acattttcaac	ctgaacctca	aataggagaa	tctcagtggt	acgaaactga	20520
aattaatcat	gcagctggga	gagtccttaa	aaagactacc	ccaatgaaac	catgttacgg	20580
ttcatatgca	aaaccacaaa	atgaaaatgg	agggcaaggc	attcttgtaa	agcaacaaaa	20640
tggaaagcta	gaaagtcaag	tggaaatgca	attttttctca	actactgagg	cgaccgcagg	20700
caatggtgat	aacttgactc	ctaaagtggg	attgtacagt	gaagatgtag	atatagaaac	20760
cccagacact	catattttctt	acatgcccac	tattaaggaa	ggtaactcac	gagaactaat	20820
gggccaacaa	tctatgcccc	acaggcctaa	ttacattgct	tttagggaca	attttattgg	20880
tctaattgtat	tacaacagca	cgggtaatat	gggtgttctg	gcgggccaag	catcgcagtt	20940
gaatgctgtt	gtagatttgc	aagacagaaa	cacagagcct	tcataccagc	ttttgcttga	21000
ttccatttgt	gatagaacca	ggtaactttc	tatgtggaat	caggctgttg	acagcttaga	21060
tcagatggt	agaattattg	aaaatcatgg	aactgaagat	gaacttccaa	attactgctt	21120
tcactgggga	ggtgtgatta	atacagagac	tcttaccaaag	gtaaaaccta	aaacagggtca	21180
ggaaaatgga	tgggaaaaag	atgctacaga	atttttcagat	aaaaatgaaa	taagagttgg	21240
aaataatttt	gcatgggaaa	tcaatctaaa	tggcaacctg	tggagaaaatt	tcctgtactc	21300
caacatagcg	ctgtattttgc	ccgacaagct	aaagtacagt	ccttccaacg	taaaaaatttc	21360
tgataaccga	aacacctacg	actacatgaa	caagcgagtg	gtggctcccc	ggttagtgga	21420
tgactacatt	aaccttgagg	cacgctgggt	ccttgactat	atggacaacg	tcaacccatt	21480
taaccaccac	cgcaatgctg	gcctgcgcta	cgctcaatg	ttgctgggca	atggctcgta	21540
tgtgcccttc	cacatccagg	tgccctcagaa	gtcttttgcc	attaaaaaac	tccttctcct	21600
gccgggctca	tacacctacg	agtggaaact	cagggaaggat	gttaacatgg	ttctgcagag	21660
ctccctagga	aatgacctaa	gggttgacgg	agccagcatt	aagtttgata	gcattttgct	21720
ttacgccacc	ttcttcccca	tggcccacaa	caccgcctcc	acgcttgagg	ccatgcttag	21780
aaacgacacc	aacgacagc	cctttaacga	ctatctctcc	gccgccaaca	tgctctacc	21840
tatacccgcc	aacgctacca	acgtgcccac	atccatcccc	tcccgaact	ggcgccgttt	21900
ccgcggtgg	gccttcacgc	gccttaagac	taaggaaacc	ccatcactgg	gctcgggcta	21960
cgacccttat	tacacctact	ctggctctat	accctaccta	gatggaacct	tttacctcaa	22020
ccacaccttt	aagaaggtgg	ccattacctt	tgactcttct	gtcagctggc	ctggcaatga	22080
ccgctgctt	accccacag	agtttgaaat	taagcgctca	gttgacgggg	aggggttaca	22140
cggtgcccg	tgtaacatga	ccaaagactg	gttctgggta	caaagtctag	ctaactacaa	22200
cattggctac	cagggtctct	atatcccaga	gagctacaag	gaccgcatgt	actccttctt	22260

-95-

tagaaacttc	cagcccatga	gocgtcaggt	ggtggatgat	actaaatata	aggactacca	22320
acaggtgggc	atcctacacc	aacacaacaa	ctctggattt	gttggctacc	ttgccccccac	22380
catgcgcgaa	ggacaggcct	acccgtctaa	cttcccctat	ccgcttatag	gcaagaccgc	22440
agttgacagc	attaccacaga	aaaagtttct	ttgcgatcgc	accctttggc	gcatcccat	22500
ctccagtaac	tttatgtcca	tgggcgcact	cacagacctg	ggccaaaacc	ttctctacgc	22560
caactccgcc	cacgcgctag	acatgacttt	tgagggtggat	cccatggacg	agcccaccct	22620
tctttatggt	ttgtttgaag	tctttgacgt	ggtccgtgtg	caccggccgc	accgcgccgt	22680
catcgaaacc	gtgtacctgc	gcacgcocct	ctcgccggc	aacgccacaa	cataaagaag	22740
caagcaacat	caacaacagc	tgccgccatg	ggctccagt	agcaggaact	gaaagccatt	22800
gtcaaagatc	ttggtttgtg	gccatatttt	ttgggcacct	atgacaagcg	ctttccaggc	22860
tttgtttctc	cacacaagct	cgcctgcgcc	atagtcaata	cggccggctc	cgagactggg	22920
ggcggtacac	ggatggcctt	tgccgtgaac	ccgcactcaa	aaacatgcta	cctctttgag	22980
ccctttggct	tttctgacca	gcgactcaag	caggtttacc	agtttgagta	cgagtcactc	23040
ctgcgccgta	gcgccattgc	ttcttcccc	gaccgctgta	taacgctgga	aaagtccacc	23100
caaagcgtac	agggggccaa	ctcgcccgcc	tgtggactat	tctgctgcat	gtttctccac	23160
gcctttgcca	actggcccca	aactcccatg	gatcacaacc	ccaccatgaa	ccttattacc	23220
ggggtaacca	actccatgct	caacagtcct	caggtacacg	ccaccctgcg	tcgcaaccag	23280
gaacagctct	acagcttctt	ggagcgccac	tcgccctact	tcgcgagcca	cagtgccgag	23340
attaggagcg	ccacttcttt	ttgtcacttg	aaaaacatgt	aaaaataatg	tactagagac	23400
acttttcaata	aaggcaaatg	cttttatattg	tacactctcg	ggtgattatt	tacccccacc	23460
cttgccgtct	gcgccgtttg	gggagggcgg	ggcgacgggg	acggggacga	cacgtcctcc	23520
atgggtgggg	gacgtcgccg	cgcaccgcgt	ccgcgctcgc	gggtgggttc	gcgctgctcc	23580
tcttcccgac	tgcccatctt	cttctcctat	aggcagaaaa	agatcatgga	gtcagtcgag	23640
aagaaggaca	gcctaaccgc	cccctctgag	ttcgccacca	ccgcctccac	cgatgccgcc	23700
aacgcgccta	ccaccttccc	cgtcgaggca	cccccgcttg	aggaggagga	agtgattatc	23760
gagcaggacc	caggttttgt	aagcgaagac	gacgaggacc	gctcagtacc	aacagaggat	23820
aaaaagcaag	accaggacaa	gcagaggcca	aacgaggaac	aagtccggcg	gggggacgaa	23880
aggcatggcg	actacctaga	tgtgggagac	gacgtgctgt	tgaagcatct	gcagcgccag	23940
tgccgccatta	tctgcgacgc	gttgcaagag	cgcagcgatg	tgccctcgc	catagcggat	24000
gtcagccttg	cctacgaacg	ccacctattc	tcaccgcgcg	taccccccaa	acgccaaaga	24060
aacgycacat	gcgagcccaa	cccgcgcctc	aacttctacc	ccgtatttgc	cgtgccagag	24120
gtgcttgcca	cctatcacat	ctttttccaa	aactgcaaga	tacccctatc	ctgcccgtgc	24180
aaccgcagcc	gagcggacaa	gcagctggcc	ttgcggcagg	gcgctgtcat	acctgatatc	24240
gcctcgctca	acgaagtgcc	aaaaatcttt	gaggggtcttg	gacgcgacga	gaagcgcgcg	24300
gcaaacgcct	tgcaacagga	aaacagcgaa	aatgaaagtc	actctggagt	gttgggtgga	24360
ctcgagggtg	acaacgcgcg	cctagccgta	ctaaaacgca	gcatcgaggt	caccacttt	24420
gcctaccggg	cacttaacct	accccccaag	gtcatgagca	cagtcatgag	tgagctgatc	24480
gtgcgccgtg	cgcagcccct	ggagagggat	gcaaatattg	aagaacaaac	agaggagggc	24540
ctaccgcgag	ttggcgacga	gcagctagcg	cgttggtctc	aaacgcgcga	gcctgccgac	24600
ttggaggagc	gacgcaaac	aatgatggcc	gcagtgcctc	ttaccgtgga	gcttgagtag	24660
atgcagcggt	tctttgctga	cccggagatg	cagcgcaagc	tagaggaaac	attgcatgac	24720
acctttcgac	agggctacgt	acgccaggcc	tgcaagatct	ccaacgtgga	gctctgcaac	24780
ctgggtctcct	accttggaat	tttgcacgaa	aaccgccttg	ggcaaaacgt	gcttcatctc	24840
acgctcaagg	gcgaggcgcg	ccgcgactac	gtccgcgact	gcgtttactt	atcttctatg	24900
tacacctggc	agacggccat	gggcgttttg	cagcagtgc	tggaggagt	caacctcaag	24960
gagctgcaga	aactgctaaa	gcaaaacttg	aaggacctat	ggacggcctt	caacgagcgc	25020
tcogtggccg	cgcacctggc	ggacatcatt	ttccccgaac	gcctgcttaa	aaccttgcaa	25080
caggggtctcg	cagacttcac	cagtcacaa	atgttgacga	actttaggaa	ctttatccta	25140
gagcgctcag	gaatcttgcc	cgccacctgc	tgtgcacttc	ctagcgactt	tgtgcccact	25200
aagtaccgcg	aatgccctcc	gccgcttttg	ggccactgct	accttctgca	gctagccaac	25260
taccttgctt	accactctga	cataatggaa	gacgtgagcg	gtgacggctc	actggagtg	25320
cactgtcgct	gcaacctatg	caccccgcac	cgtccctgg	tttgcaattc	gcagctgctt	25380
aacgaaagtc	aaattatcgg	tacctttgag	ctgcagggtc	cctcgcttga	cgaaaagtc	25440
gcggctccgg	ggttgaaact	cactccgggg	ctgtggacgt	cggcttacct	tcgcaaatct	25500
gtacctgagg	actacacgc	ccacgagatt	aggttctacg	aagaccaatc	ccgcccgcga	25560
aatgcggagc	ttaccgcctg	cgtcattacc	cagggccaca	ttcttggcca	attgcaagcc	25620
atcaacaaaag	cccgccaaga	gtttctgcta	cgaaagggac	gggggggtta	cttggaaccc	25680
cagtcggcgg	aggagctcaa	cccaatcccc	ccgcgcgcgc	agccctatca	gcagcagccg	25740
cgggcccttg	cttccacgga	tgccacccaa	aaagaagctg	cagctgccc	cgccaccac	25800
ggacgaggag	gaatactggg	acagtcaggc	agaggaggtt	ttggacgagg	aggaggagga	25860
catgatggaa	gactgggaga	gcctagacga	ggaagcttcc	gaggtcgaag	aggtgtcaga	25920

-96-

cgaaacaccg	tcaccctcgg	tgcatttccc	ctcgccggcg	ccccagaaat	cggcaaccgg	25980
ttccagcatg	gctacaacct	cgcgtcctca	ggcgccggcg	gcactgcccg	ttcgccgacc	26040
caaccgtaga	tgggacacca	ctggaaccag	ggccggtaag	tccaagcagc	cgccgcggtt	26100
agcccaagag	caacaacagc	gccaaggcta	ccgctcatgg	cgccggcaca	agaacgccat	26160
agttgcttgc	ttgcaagact	gtgggggcaa	catctccttc	gcccgcgct	ttcttctcta	26220
ccatcacggc	gtggccttcc	cccgtaacat	cctgcattac	taccgtcatc	tctacagccc	26280
atactgcacc	ggcggcagcg	gcagcggcag	caacagcagc	ggccacacag	aagcaaaggc	26340
gaccggatag	caagactctg	acaaagccca	agaaatccac	agcggcggca	gcagcaggag	26400
gaggagcgct	gcgtctggcg	cccaacgaac	ccgtatcgac	ccgcgagctt	agaaacagga	26460
tttttcccac	tctgtatgct	atattttcaac	agagcagggg	ccaagaacaa	gagctgaaaa	26520
taaaaaacag	gtctctgcga	tccctcaccc	gcagctgcct	gtatcacaaa	agcgaagatc	26580
agcttcggcg	cacgctggaa	gacgcggagg	ctctcttcag	taaatactgc	gcgctgactc	26640
ttaaggacta	gtttcgcgcc	ctttctcaaa	tttaagcgcg	aaaactacgt	catctccagc	26700
ggccacaccc	ggcgccagca	cctgtcgtca	gcgccattat	gagcaaggaa	attccccagc	26760
cctacatgtg	gagttaccag	ccacaaatgg	gacttgccgg	tggagctgcc	caagactact	26820
caaccggaat	aaactacatg	agcgcgggag	cccacatgat	atcccgggtc	aacggaatcc	26880
gcgcccaccg	aaaccgaatt	ctcttggaac	aggcgctat	taccaccaca	cctcgtaata	26940
accttaatcc	ccgtagtgtg	cccgtgccc	tggtgtacca	ggaaagtccc	gctcccacca	27000
ctgtggtact	tcccagagac	gcccaggccg	aagttcagat	gactaactca	ggggcgagc	27060
ttgcggggcg	ctttcgtcac	agggtgcggt	cgcccgggca	gggtataact	cacctgacaa	27120
tcagagggcg	aggtattcag	ctcaacgacg	agtcggtgag	ctcctcgctt	ggtctccgtc	27180
cggacgggac	atttcagatc	ggcggcgccg	gcccgtcctt	attcacgcct	cgtcaggcaa	27240
tcctaactct	gcagacctcg	tcctctgagc	cgcgctctgg	aggcattgga	actctgcaat	27300
ttattgagga	gtttgtgcca	tcggtctact	ttaacccctt	ctcgggacct	cccggccact	27360
atccggatca	atttattcct	aactttgacg	cggtaaaggga	ctcgggcgac	ggctacgact	27420
gaatgttaag	tggagaggca	gagcaactgc	gcttgaaaca	cctgggtccac	tgtcgccgcc	27480
acaagtgcct	tgcccgcgac	tcgggtgagt	tttgtactt	tgaattgccc	gaggatcata	27540
tcgagggccc	ggcgcacggc	gtccggctta	ccgccagggg	agagcttgcc	cgtagcctga	27600
ttcgggagtt	taccagcgcc	cccctgctag	ttgagcggga	caggggaccc	tgtgttctca	27660
ctgtgatttg	caactgtcct	aaccttggat	tacatcaaga	tctttgttgc	catctctgtg	27720
ctgagtataa	taaatacaga	aattaaaata	tactggggct	cctatcgcca	tcctgtaaac	27780
gccaccgtct	tcaccgcgcc	aagcaaacca	aggcgaacct	tacctggtac	ttttaacatc	27840
tctccctctg	tgattttacaa	cagtttcaac	ccagacggag	tgagtctacg	agagaacctc	27900
tcogagctca	gctactccat	cagaaaaaac	accaccctcc	ttacctgccg	ggaacgtacg	27960
agtgcgtcac	cggccgctgc	accacaccta	ccgcctgacc	gtaaaccaga	ctttttccgg	28020
acagacctca	ataactctgt	ttaccagaac	aggaggtgag	cttagaaaaa	ccttagggta	28080
ttaggccaaa	ggcgagctta	ctgtgggggt	tatgaacaat	tcaagcaact	ctacgggcta	28140
ttctaattca	ggtttctcta	gaaatggacg	gaattattac	agagcagcgc	ctgctagaaa	28200
gacgcagggc	agcggccgag	caacagcgca	tgaatcaaga	gctccaagac	atggttaact	28260
tgcaccagtg	caaaaagggg	atcttttgtc	tggttaagca	ggccaaagtc	acctacgaca	28320
gtaataccac	cggacaccgc	ccttagctaca	agttgccaac	caagcgtcag	aaattgggtg	28380
tcatggtggg	agaaaagccc	attaccataa	ctcagcactc	ggtagaaacc	gaaggctgca	28440
ttcactcacc	ttgtcaagga	cctgaggatc	tctgcaccct	tattaagacc	ctgtgcgggtc	28500
tcaaagatct	tattcccttt	aactaataaa	aaaaaataat	aaagcatcac	ttacttaaaa	28560
tcagttagca	aattttctgtc	cagttttattc	agcagcacct	ccttgccctc	ctccagctc	28620
tggtattgca	gcttcctcct	ggctgcaaac	tttctccaca	atctaaatgg	aatgtcagtt	28680
tcctcctgtt	cctgtccatc	cgcaccact	atcttcatgt	tgttgcagat	gaagcgcgca	28740
agaccgtctg	aagatacctt	caaccccggt	tatccatatg	acacggaaac	cggctcctcca	28800
actgtgcctt	ttcttactcc	tccctttgta	tcccccaatg	ggtttcaaga	gagtcgccct	28860
gggtgactct	ctttgcgcct	atccgaacct	gctgttacct	ccaatggcat	gcttgcgctc	28920
aaaatgggca	acggcctctc	tctggacgag	gcccggcaacc	ttacctccca	aaatgtaacc	28980
actgtgagcc	cacctctcaa	aaaaaccaag	tcaaacataa	acctggaaat	atctgcaccc	29040
ctcacagtta	cctcagaagc	cctaactgtg	gctgccggcg	cacctctaata	ggtcgcgggc	29100
aacacaccca	acaggccccc	agtgtcagaa	ctaaccgtgc	acgactccaa	acttagcatt	29160
gccacccaag	gacccctcac	tacccttact	ggaaagctag	ccctgcaaac	atcaggcccc	29220
ctcaccacca	ccgatagcag	cttgaaagag	atcactgcct	cacccctctc	aactactgcc	29280
actggtagct	tgggcattga	gcatgtaaca	cccattttata	cacaaaatgg	aaaactagga	29340
ctaaagtacg	gggctccttt	taatacttcc	gacgacctaa	acactttgac	cgtagcaact	29400
gggtccaggtg	tgactattaa	gcaacttaat	ttgcaaaacta	aagttactgg	agccttgggt	29460
tttgattcac	aaggcaatat	tgtagttagt	gtagcaggag	gactaaggat	tgattctcaa	29520
aacagacgcc	ttatacttga		ccgtttgatg	ctcaaaacca	actaaatcta	29580

-97-

agactaggac	agggccctct	ttttataaac	tcagcccaca	acttggatat	taactacaac	29640
aaaggccttt	acttgtttac	agcttcaaac	aattccaaaa	agcttgagggt	taacctaaagc	29700
actgccaaagg	ggttgatggt	tgacgctaca	gccatagcca	ttaatgcagg	agatgggctt	29760
gaatttggtt	cacctaattgc	accaaacaca	aatccctca	aaacaaaaat	tggccatggc	29820
ctagaatttg	attcaaacaa	ggctatgggt	cctaaactag	gaactggcct	tagttttgac	29880
agcacagggtg	ccattacagt	aggaaacaaa	aataatgata	agctaaacttt	gtggaccaca	29940
ccagctccat	ctcctaactg	tagactaaat	gcagagaaaag	atgctaaact	cacttttggtc	30000
ttaacaaaat	gtggcagtc	aatacttgct	acagttttcag	ttttggctgt	taaaggcagt	30060
ttggctccaa	tatctggaac	agttcaaagt	gctcatctta	ttataagatt	tgacgaaaaat	30120
ggagtgcctac	taaacaattc	cttcctggac	ccagaatatt	ggaacttttag	aatggagat	30180
cttactgaag	gcacagccta	tacaaacgct	gttggattta	tgccctaacct	atcagcttat	30240
ccaaaatctc	acggtaaaac	tgccaaaagt	aacattgtca	gtcaagttta	cttaaacgga	30300
gacaaaacta	aacctgtaac	actaaccatt	acactaaacg	gtacacagga	aacaggagac	30360
acaactccaa	gtgcatactc	tatgtcattt	tcatgggact	ggtctggcca	caactacatt	30420
aatgaaatat	ttgccacatc	ctcttacact	ttttcataca	ttgcccaaga	ataaagaatc	30480
gtttgtggtta	tgtttcaacg	tgtttatttt	tcaattgcag	aaaatttcaa	gtcattttttc	30540
attcagtagt	atagcccac	caccacatag	cttatacaga	tcaccgtacc	ttaactaaaat	30600
tcacagaacc	ctagtattca	acctgccacc	tcctcccaa	cacacagagt	acacagtcct	30660
ttctccccgg	ctggccttaa	aaagcatcat	atcatgggta	acagacatat	tcttaggtgt	30720
tatattccac	acggtttctt	gtcgagccaa	acgctcatca	gtgatattaa	taaactcccc	30780
gggcagcttc	cttaagttca	tgctcgctgtc	cagctgctga	gccacaggct	gctgtccaac	30840
ttgcggttgc	ttaacgggcg	gcgaaggaga	agtccacgcc	tacatggggg	tagagtcata	30900
atcgtgcatc	aggatagggc	ggtggtgctg	cagcagcgcg	cgaataaaact	gctgccgcgcg	30960
ccgctccgtc	ctgcaggaat	acaacatggc	agtggctctcc	tcagcgatga	ttcgccaccgc	31020
ccgcagcata	aggcgcttg	tcctccgggc	acagcagcgc	acctgatct	cacttaaatc	31080
agcacagtaa	ctgcagcaca	gcaccacaat	attgttcaaa	atcccacagt	gcaaggcgct	31140
gtatccaaag	ctcatggcgg	ggaccacaga	acccacgtgg	ccatcatacc	acaagcgcgag	31200
gtagattaag	tgccgacccc	tcataaacac	gctggacata	aacattacct	cttttggcat	31260
gttgtaattc	accacctccc	ggtaccatat	aaacctctga	ttaaacatgg	cgccatccac	31320
caccatccta	aaccagctgg	ccaaaacctg	cccgcgggct	atacactgca	gggaaccggg	31380
actggaacaa	tgacagtgga	gagcccagga	ctcgtaacca	tggatcatca	tgctcgctcat	31440
gatatcaatg	ttggcacaaac	acaggcacac	gtgcatacac	ttcctcagga	ttacaagctc	31500
ctcccgcggt	agaaccatat	cccagggaac	aaccttctcc	tgaatcagcg	taaatcccac	31560
actgcaggga	agacctcgca	cgtaactcac	gttggtgcatt	gtcaaagtgt	tacattcggg	31620
cagcagcgga	tgatcctcca	gtatggtagc	gcgggtttct	gtctcaaaag	gaggtagacg	31680
atccctactg	tacggatgct	gccgagacaa	ccgagatcgt	gttggtcgta	gtgtcatgcc	31740
aaatggaacg	ccggacgtag	tcataatttcc	tgaagcaaaa	ccagggtcg	gcgtgacaaa	31800
cagatctgcg	tctccggtct	cgccgcttag	atcgctctgt	gtagtagttg	tagtatatcc	31860
actctctcaa	agcatccagg	cgccccctgg	cttcgggttc	tatgtaaact	ccttcatgcg	31920
ccgctgcctc	gataacatcc	accacgcgag	aataagccac	accagccaa	cctacacatt	31980
cgctctcgca	gtcacacacg	ggaggagcgg	gaagagctgg	aagaaccatg	tttttttttt	32040
tattccaaaa	gattatccaa	aacctcaaaa	tgaagatcta	ttaagtgaac	gcgctccctc	32100
ccggtggcgt	ggtcaaaactc	tacagccaaa	gaacagataa	tggcattttgt	aagatgttgc	32160
acaatggctt	ccaaaaggga	aacggccctc	acgtccaagt	ggacgtaaa	gctaaacctt	32220
tcagggtgaa	tctcctctat	aaacattcca	gcaccttcaa	ccatgcccaa	ataattctca	32280
tctcgccacc	ttctcaatat	atctctaagc	aaatcccgaa	tattaagtcc	ggccatttga	32340
aaaatctgct	ccagagcgcc	ctccaccttc	agcctcaagc	agcgaatcat	gattgcaaaa	32400
attcagggttc	ctcacagacc	tgtataagat	tcaaaaagcgg	aacattaaca	aaaataccgc	32460
gatcccgtag	gtcccttctgc	agggccagct	gaacataatc	gtgcaggctc	gcacggacca	32520
gcgcggccac	ttccccgcga	ggaaccttga	caaaagaacc	cacactgatt	atgacacgca	32580
tactcgagac	tatgctaacc	agcgtagccc	cgatgtaagc	tttgttgcat	ggggcgcgat	32640
ataaaatgca	aggtgctgct	caaaaaatca	ggcaaagcct	cgcgcaaaaa	agaaagcaca	32700
tcgtagtcat	gctcatgcag	ataaaggcag	gtaagctccg	gaaccaccac	agaaaaagac	32760
accatttttc	tctcaatgac	gtctgcggtg	ttctgcataa	acacaaaata	aaataacaaa	32820
aaaacattta	aacattagaa	gcctgtctta	caacaggaaa	aacaaccctt	ataagcataa	32880
gacggactac	ggccatgccg	gcgtgaccgt	aaaaaaactg	gtcaccgtga	ttaaaaagca	32940
ccaccgacag	ctcctcggtc	atgtccggag	tcataatgta	agactcggtg	aacacatcag	33000
gttgattcat	cggtcagtcg	taaaaagcga	ccgaaatagc	ccgggggaat	acatacccg	33060
aggcgtagag	acaacattac	agccccata	ggagggtataa	caaaattaat	aggagagaaa	33120
aacacataaa	cacctgaaaa	acctcctgc	ctaggcaaaa	tagcacctc	ccgctccaga	33180
acaacataca	gcgcttcaca	gcggcagcct	aacagtcagc	cttaccagta	aaaaagaaaa	33240

-98-

```

cctattaaaa aaacaccact cgacacggca ccagctcaat cagtcacagt gtaaaaaagg 33300
gccaaagtga gagcgagtat atataggact aaaaaaatgac gtaacgggta aagtccacaa 33360
aaaaacacca gaaaaccgca cgcgaaaccta cgcccagaaa cgaaagccaa aaaacccaca 33420
acttcctcaa atcgctcactt ccgttttccc acgttacgta acttcccatt ttaagaaaac 33480
tacaattccc aacacataca agttactccg ccctaaaacc tacgtcaccg gcccgcgttcc 33540
cacgccccgc gccacgtcac aaactccacc ccctcattat catattggct tcaatccaaa 33600
ataaggatata ttattgatga tg                                     33622

```

<210> 53

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 5FF

<400> 53

gaacaggagg tgagcttaga

20

<210> 54

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 5FR

<400> 54

tccgcctcca tttagtgaac agttaggaga tggagctggg gtg

43

<210> 55

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 3FF

<400> 55

tcactaaatg gaggcggaga tgctaaactc actttgggtct taac

44

<210> 56

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 3FR

<400> 56

gtggcagggtt gaatactagg

20

<210> 57

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Penton 1 Oligonucleotide

<400> 57

-99-

cgcggaagag aactccaacg cggcagccgc ggcaatgcag ccggtggagg acatgaa 57

<210> 58

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> Penton 2 Oligonucleotide

<400> 58

tatcggttcac gtcctccacc ggctgcattg ccgcggctgc cgcgttgagg ttctcttcc 59

<210> 59

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Penton 3 Oligonucleotide

<400> 59

cgatagccgc ggctaccctt acgacgtgcc cgactacgcg ggcaccagcg ccacacgggc 60
tgaggagaag cgcgc 75

<210> 60

<211> 73

<212> DNA

<213> Artificial Sequence

<220>

<223> Penton 4 Oligonucleotide

<400> 60

tcagcgcgct tctcctcagc ccgtgtggcg ctgggtgcccg cgtagtcggg cacgtcgtag 60
gggtagccgc ggc 73

<210> 61

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Hexon Forward Primer

<400> 61

cttcgatgat gccgcagtg 19

<210> 62

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Hexon Reverse Primer

<400> 62

gggctcaggt actccgagg 19

<210> 63

<211> 25

-100-

<212> DNA
<213> Artificial Sequence

<220>
<223> Hexon Probe

<400> 63
ttacatgcac atctcgggcc aggac

25

<210> 64
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> 5HSPR primer

<400> 64
ggctccggct ccgagaggtg ggctcacagt ggttacattt

40

<210> 65
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> P-0005/U primer

<400> 65
ctctagaaat ggacggaatt attacag

27

<210> 66
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> P-0006/L primer

<400> 66
tcttgggtcat ctgcaacaac atgaagatag tg

32

<210> 67
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> P-0007/U primer

<400> 67
gttggtgcag atgaccaaga gagtccggct ca

32

<210> 68
<211> 73
<212> DNA
<213> Artificial Sequence

<220>
<223> 35FMun primer

-101-

<400> 68
agcaattgaa aaataaacac gttgaaacat aacacaaacg attcttttagt tgtcgtcttc 60
tgtaatgtaa gaa 73

<210> 69
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> P-0009 primer

<400> 69
agcaattgaa aaataaacac gttg 24

<210> 70
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer P1

<400> 70
gaacaggagg tgagcttaga 20

<210> 71
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer P2

<400> 71
gttaggtgga gggttttattc cgggtccacaa agttagctta tc 42

<210> 72
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer P3

<400> 72
gataagctaa ctttgtggac cggaataaac cctccaccta ac 42

<210> 73
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer P4

<400> 73
gtggcagggtt gaataactagg 20

<210> 74
<211> 41

-102-

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer P5

<400> 74
 gttaggagat ggagctgggtg tagtcataa ggtgttaata c 41

<210> 75
 <211> 41
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer P6

<400> 75
 gtattaacac cttatggact acaccagctc catctcctaa c 41

<210> 76
 <211> 54
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer P7

<400> 76
 tgcgcaaaaa caatcaccac gacaatcaca atgtacattg gaagaaatca tacg 54

<210> 77
 <211> 54
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer P8

<400> 77
 acattgtgat tgcgtgggtg attgtttttg cgcataatgcc atacaatttg aatg 54

<210> 78
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> cRGD peptide

<400> 78
 His Cys Asp Cys Arg Gly Asp Cys Phe Cys
 1 5 10

<210> 79
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>

-103-

<223> P-0010/L primer

<400> 79

ttctttttcat ctgcaacaac atgaagatag tg

32

<210> 80

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> P-0011/U primer

<400> 80

gttggtgcag atgaaaagaa ccagaattga ag

32

<210> 81

<211> 73

<212> DNA

<213> Artificial Sequence

<220>

<223> P-0012/L primer

<400> 81

tgcaattgaa aaataaacac gttgaaacat aacacaaacg attctttatt cttcagttat 60
gtagcaaaat aca 73

<210> 82

<211> 56

<212> DNA

<213> Artificial Sequence

<220>

<223> 41sRGDR Primer

<400> 82

agtacaaaaa caatcaccac gacaatcaca gtttatctcg ttgtagacga cactga 56

<210> 83

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> 41sRGDF Primer

<400> 83

tgtgattgtc gtggtgattg tttttgtact agtgggtatg cttttacttt t 51

<210> 84

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> L37 Primer

<400> 84

tgtcttggat ccaagatgaa gcgcgcgcgc cccagcgaag atgacttc 48

-104-

<210> 85
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 37FR Primer

<400> 85
 aaacacggcg gccgctcttt cattcttg

28

<210> 86
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Native Ad37 N-terminus

<400> 86
 Met Ser Lys Arg Leu Arg Val Glu
 1 5

<210> 87
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified Ad37 N-terminus

<400> 87
 Met Lys Arg Ala Arg Pro Ser Glu
 1 5

<210> 88
 <211> 1240
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad5 TPL sequence

<400> 88
 ggatccactc tcttccgcat cgctgtctgc gagggccagc tgttgggggtg agtactccct 60
 ctgaaaagcg ggcattgactt ctgcgctaag attgtcagtt tccaaaaacg aggaggatttt 120
 gatattcacc tggcccgcgg tgatgccttt gaggggtggcc gcatccatct ggtcagaaaa 180
 gacaatcttt ttgttgtcaa gcttgggtggc aaacgaccgc tagagggcgt tggacagcaa 240
 cttggcgatg gagcgcagggt tttgggtttt gtcgcgatcg gcgcgctcct tggccgcgat 300
 gtttagctgc acgtattcgc gcgcaacgca ccgccattcg ggaaagacgg tgggtgcgctc 360
 gtcgggcacc aggtgcacgc gccaaaccgc gttgtgcagg gtgacaagggt caacgcctgg 420
 ggctacctct ccgcgtaggc gctcgttggg ccagcagagg cggccgcctt tgcgcgagca 480
 gaatggcggt aggggggtcta gctgcgtctc gtccgggggg tctgcgtcca cggtaaaagac 540
 cccgggacgc aggcgcgcgt cgaagtagtc tatcttgcac ccttgcaagt ctagegcctg 600
 ctgccatgcg cgggcggcaa gcgcgcgctc gtatgggttg agtgggggac cccatggcat 660
 ggggtgggtg agcgcggagg cgtacatgcc gcaaagtgcg taaacgtaga ggggctctct 720
 gagtattcca agatatgtag ggtagcatct tccaccgcgg atgctggcgc gcacgtaatc 780
 gtatagttcg tgcgaggagg cgaggaggtc gggaccgagg ttgctaaggg cgggctgctc 840
 tgctcggaag actatctgcc tgaagatggc atgtgagttg gatgatattg ttggacgctg 900

-105-

```

gaagacgttg aagctggcgt ctgtgagacc taccgcgtca cgcacgaagg aggcgtagga 960
gtcgcgcagc ttgttgacca gctcggcggg gacctgcacg tctagggcgc agtagtccag 1020
ggtttccttg atgatgtcat acttatcctg tccctttttt ttccacagct cgcgggttgag 1080
gacaaactct tcgcgggtctt tccagtactc ttggatcgga aaccgcgtcg cctccgaacg 1140
agatccgtac tccgcgcgcg agggacctga gcgagtcgcg atcgaccgga tcggaaaacc 1200
tctcgagaaa ggcgtctaac cagtcacagt cgcaagatct 1240

```

<210> 89
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer F16 5'

<400> 89
 ccggtctacc catatgaaga tg 22

<210> 90
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer F16 3'

<400> 90
 tggtgcggcc gctcagtcac cttctctg 28

<210> 91
 <211> 34
 <212> DNA
 <213> qArtificial Sequence

<220>
 <223> Primer F35 3'

<400> 91
 tggtgcggcc gcttagttgt cgtcttctgt aatg 34

<210> 92
 <211> 10837
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Plasmid p5FloxHRF

<400> 92
 ggaatacaac atggcagtg tctcctcagc gatgattcgc accgcccgc gcataaggcg 60
 ccttgcctc cgggcacagc agcgcaccct gatctcactt aaatcagcac agtaactgca 120
 gcacagcacc acaatattgt tcaaaatccc acagtgcag gcgctgtatc caaagctcat 180
 ggcggggacc acagaaccca cgtggccatc ataccacaag cgcaggtaga ttaagtggcg 240
 accctcata aacacgttg acataaacat tacctctttt ggcatgttgt aattcaccac 300
 ctcccggtac catataaacc tctgattaaa catggcgcca tccaccacca tcctaaacca 360
 gctggccaaa acctgccgc cggctatata ctgcagggaa ccgggactgg aacaatgaca 420
 gtggagagcc caggactcgt aaccatggat catcatgctc gtcagatgat caatgttggc 480
 acaacacagg cacacgtgca tacacttcct caggattaca agctcctccc gcgttagaac 540
 catatcccag ggaacaaccc attcctgaat cagcgtaaat ccacactgc aggggaagacc 600
 tcgcacgtaa ctcacgttgt gcattgtcaa agtggtacat tcgggcagca gcggatgatc 660
 ctccagtatg gtagcgcggg tttctgtctc aaaaggagggt agacgatccc tactgtacgg 720

-106-

agtgcgccga	gacaaccgag	atcgtgttgg	tcgtagtgtc	atgccaaatg	gaacgccgga	780
cgtagtcata	tttctgaag	caaaaaccagg	tgcggggcgtg	acaaacagat	ctgcgtctcc	840
ggctctgccg	cttagatcgc	tctgtgtagt	agttgtagta	tatccactct	ctcaaagcat	900
ccaggcgccc	cctggcttcg	ggttctatgt	aaactccttc	atgcgcgcgt	gcoctgataa	960
catccaccac	cgcagaataa	gccacacca	gccaacctac	acattcgttc	tgcgagtcac	1020
acacggggagg	agcgggaaga	gctggaagaa	ccatgttttt	ttttttatcc	caaaagatta	1080
tccaaaacct	caaaatgaag	atctattaag	tgaacgcgct	cccctccggt	ggcgtgggtca	1140
aactctacag	ccaaagaaca	gataatggca	tttgtaaagt	gttgacacat	ggcttccaaa	1200
aggcaaacgg	ccctcacgtc	caagtggacg	taaaggctaa	acccttcagg	gtgaatctcc	1260
tctataaaca	ttccagcacc	ttcaaccatg	cccaaataat	tctcatctcg	ccaccttctc	1320
aatatatctc	taagcaaato	ccgaatatta	agtccggcca	ttgtaaaaat	ctgctccaga	1380
gcccctccca	ccttcagcct	caagcagcga	atcatgattg	caaaaaattca	ggttcctcac	1440
agacctgtat	aagattcaaa	agcgggaacat	taacaaaaat	accgcgatcc	cgtaggctccc	1500
ttcgcagggc	cagctgaaca	taatcgtgca	ggctctgcacg	gaccagcgcg	gccacttccc	1560
cgccaggaac	cttgacaaaa	gaacccacac	tgattatgac	acgcatactc	ggagctatgc	1620
taaccagcgt	agccccgatg	taagctttgt	tgcattggcg	gcatataaaa	atgcaagggtg	1680
ctgctcaaaa	aatcaggcaa	agcctcgcgc	aaaaaagaaa	gcacatcgta	gtcatgctca	1740
tgcagataaa	ggcaggtaag	ctccggaacc	accacagaaa	aagacaccat	tttctctca	1800
aacatgtctg	cgggtttctg	cataaacaca	aaataaaata	acaaaaaac	atttaaacad	1860
tagaagcctg	tcttacaaca	ggaaaaacaa	cccttataag	cataagacgg	actacggcca	1920
tgcggcgctg	accgtaaaaa	aactggtcac	cgtgattaaa	aagcaccacc	gacagctcct	1980
cggctatgtc	cggagtcata	atgtaagact	cggtaaacac	atcagggtga	ttcatcggtc	2040
agtgtctaaa	agcgaccgaa	atagcccggg	ggaatacata	cccgcaggcg	tagagacaac	2100
attacagccc	ccataggagg	tataacaaaa	ttaataggag	agaaaaacac	ataaacacct	2160
gaaaaaccct	cctgcctagg	caaaatagca	ccctcccgtc	ccagaacaac	atacagcgct	2220
tcacagcggc	agcctaacag	tcagccttac	cagtaaaaaa	gaaaacctat	taaaaaaaca	2280
ccactcgaca	cggcaccagc	tcaatcagtc	acagtgtaaa	aaagggccaa	gtgcagagcg	2340
agtatatata	ggactaaaaa	atgacgtaac	ggttaaagtc	cacaaaaaac	accagaaaaa	2400
ccgcacgcga	acctacgccc	agaaacgaaa	gccaaaaaac	ccacaacttc	ctcaaatcgt	2460
cacttccggt	ttcccacggt	acgtcacttc	ccattttaat	taagaaaaact	acaattccca	2520
acacatacaa	gttactccgc	cctaaaacct	acgtcacccg	cccgtttccc	acgcccgcg	2580
ccacgtcaca	aactccaccc	cctcattatc	atatgtgctt	caatccaaaa	taagggtatat	2640
tattgatgat	ggatcagctt	atcgataccg	tcgacctcga	ggggggggccc	ggtaccaaat	2700
tcgccctata	gtgagtcgta	ttacaattca	ctggccgctc	ttttacaacg	tcgtgactgg	2760
gaaaaccctg	gcgttaacca	acttaatcgc	cttgacgac	atcccccttt	cgccagctgg	2820
cgtaatagcg	aagaggcccg	caccgatcgc	ccttcccaac	agttgcgcag	cctgaatggc	2880
gaatggcgcg	acgcgcctcg	tagcggcgca	ttaaagcgcg	cgggtgtggg	ggttacgcgc	2940
agcgtgaccg	ctacacttgc	cagcgcctta	gcgcgcgctc	ctttcgcttt	cttcccttcc	3000
tttctcgcca	cgttcgcggg	ctttccccgt	caagctctaa	atcggggggt	cccttttaggg	3060
ttccgattta	gtgctttacg	gcacctcgac	ccccaaaaac	ttgattaggg	ttaggttca	3120
cgtagtgggc	catcgccctg	atagacggtt	tttcgccttt	tgacgttggg	gtccacgttc	3180
tttaaatagt	gactcttggt	ccaaactgga	acaacactca	accctatctc	ggtctattct	3240
tttgatttat	aagggatttt	gccgatttcg	gcctattggg	taaaaaatga	gctgatttaa	3300
caaaaattta	acgcgaattt	taacaaaaata	ttaacgttta	caattttccc	ggtggcactt	3360
ttcggggaaa	tgtgcgcgga	acccctattt	gtttattttt	ctaaatacat	tcaaatatgt	3420
atccgctcat	gagacaataa	ccctgataaa	tgcttcaata	atattgaaaa	aggaagagta	3480
tgagtattca	acattttccgt	gtcgccctta	ttcccttttt	tgccggcattt	tgcccttccg	3540
tttttgctca	cccagaaacg	ctgggtgaaag	taaaagatgc	tgaagatcag	ttgggtgcac	3600
gagttgggtta	catcgaactg	gatctcaaca	gcggtaagat	ccttgagagt	tttcgccccg	3660
aagaacggtt	tccaatgatg	agcactttta	aagttctgct	atgtggcgcg	gtattatccc	3720
gtattgacgc	cgggcaagag	caactcggtc	gccgcataca	ctattctcag	aatgacttgg	3780
ttgagtactc	accagtcaca	gaaaagcatc	ttacggatgg	catgacagta	agagaattat	3840
gcagtgtctg	cataaccatg	agtgataaca	ctgcggccaa	cttacttctg	acaacgatcg	3900
gaggaccgaa	ggagctaacc	gcttttttgc	acaacatggg	ggatcatgta	actcgccttg	3960
atcgttggga	accggagctg	aatgaagcca	taccaaacga	cgagcgtgac	accacgatgc	4020
ctgtagcaat	ggcaacaacg	ttgcgcaaac	tattaactgg	cgaactactt	actctagctt	4080
cccggcaaca	attaatagac	tggatggagg	cggataaagt	tgcaggacca	cttctgcgct	4140
cggcccttcc	ggctggctgg	tttattgctg	ataaatctgg	agccgggtgag	cgtgggtctc	4200
cggtatcatc	tgcagactgt	gggcccagct	gtaagccctc	ccgtatcgta	gttatctaca	4260
cgacggggag	tcaggcaact	atggatgaac	gaaatagaca	gatcgctgag	ataggtgcct	4320
cactgattaa	gcatttggtta	ctgtcagacc	aagtttactc	atatatactt	tagattgatt	4380

-107-

taaaacttca	tttttaattt	aaaaggatct	aggtgaagat	cctttttgat	aatctcatga	4440
ccaaaatccc	ttaacgtgag	ttttcgttcc	actgagcgtc	agaccccgtg	gaaaagatca	4500
aaggatcttc	ttgagatcct	ttttttctgc	gcgtaatctg	ctgcttgcaa	acaaaaaac	4560
caccgctacc	agcgggtggt	tgtttgccgg	atcaagagct	accaactcct	tttccgaagg	4620
taactggctt	cagcagagcg	cagataccaa	atactgtcct	tctagtgtag	ccgtagttag	4680
gccaccactt	caagaactct	gtagcaccgc	ctacatacct	cgctctgcta	atcctgttac	4740
cagtggctgc	tgccagtggc	gataagtcgt	gtcttaccgg	gttggactca	agacgatagt	4800
taccggataa	ggcgagcg	tcgggctgaa	cggtttcc	gtgcacacag	cccagcttgg	4860
agcgaacgac	ctacaccgaa	ctgagatacc	tacagcgtga	gctatgagaa	agcgccacgc	4920
ttcccgaagg	gagaaaggcg	gacaggtatc	cggttaagcg	cagggtcgga	acaggagagc	4980
gcacgagggg	gcttccaggg	ggaaacgcct	ggtatcttta	tagtcctgtc	gggtttcgcc	5040
acctctgact	tgagcgtcga	tttttctgat	gctcgtcagg	ggggcgagc	ctatggaaaa	5100
acgccagcaa	cgcgcccttt	ttacggttcc	tggccttttg	ctggcctttt	gtcacatgt	5160
tctttcctgc	gttatccctt	gattctgtgg	ataaccgtat	taccgccttt	gagtgtgctg	5220
ataccgctcg	ccgcagccga	acgaccgagc	gcagcagagc	agtgtgagcg	gaagcggaag	5280
atcgcccaat	acgcaaacgg	cctctccccg	cgcgctggcc	gattcattaa	tgcagctggc	5340
acgacaggtt	tcccgaactg	aaagcgggca	gtgagcgcaa	cgcaattaat	gtgagtttag	5400
tcactcatta	ggcaccaccg	gctttacact	ttatgcttcc	ggctcgtatg	ttgtgtggaa	5460
ttgtgagcgg	ataacaattt	cacacaggaa	acagctatga	ccatgattac	gccaagctcg	5520
gaattaaccc	tcactaaagg	gaacaaaagc	tggagctcca	ccgcggggcc	ttgcttccca	5580
ggatggcacc	caaaaagaag	ctgcagctgc	cgccgcccac	cacggacgag	gaggaatact	5640
gggacagtca	ggcagaggag	gttttggagc	aggagaggga	ggacatgatg	gaagactggg	5700
agagcctaga	cgaggaagct	tccgaggtcg	aagaggtgtc	agacgaaaca	ccgtcacccct	5760
cggtcgcatt	cccctcgccg	gcgccccaga	aatcggcaac	cggttccagc	atggctacaa	5820
cctccgctcc	tcaggcgccg	ccggcactgc	ccgttcgccc	acccaaccgt	agatgggaca	5880
ccactggaa	cagggccggg	aagtccaagc	agccgcccgc	gttagcccaa	gagcaacaac	5940
agcgccaagg	ctaccgctca	tggcgcgggc	acaagaacgc	catagtgtgt	tgcttgcaag	6000
actgtggggg	caacatctcc	ttcgcccggc	gctttcttct	ctaccatcac	ggcggtggcct	6060
tcccccgtaa	catcctgcat	tactaccgtc	atctctacag	cccatactgc	accggcgggc	6120
gcggcagcgg	cagcaacagc	agcggccaca	cagaagcaaa	ggcgaccgga	tagcaagact	6180
ctgacaaagc	ccaagaaatc	cacagcgccg	gcagcagcag	gaggaggagc	gctgcgtctg	6240
gcgccccaac	aaccgcgtatc	gacccgcgag	cttagaaaca	ggatttttcc	cactctgtat	6300
gctatatttt	aacagagcag	gggccaagaa	caagagctga	aaataaaaaa	caggtctctg	6360
cgatccctca	cccgcagctg	cctgtatcac	aaaagcgaag	atcagcttcc	gcgcacgctg	6420
gaagacgcgg	aggctctctt	cagtaaatca	tgcgcgtcga	ctcttaagga	ctagtttcgc	6480
gccctttctg	aaatttaagc	gcgaaaacta	cgctactctc	agcggccaca	cccggcgcca	6540
gcacctgtcg	tcagcgccat	tatgagcaag	gaaattccca	cgccctacat	gtggagttac	6600
cagccacaaa	tgggacttgc	ggctggagct	gcccaagact	actcaaccgc	aataaactac	6660
atgagcgcgg	gacccacat	gatatcccg	gtcaacggaa	tccgcgcccc	ccgaaaccga	6720
attctcctgg	aacagcgccg	tattaccacc	acacctcgta	ataaccttaa	tcccgcctag	6780
tggcccgcgt	ccctggtgta	ccaggaaagt	cccgtctcca	ccactgtggt	acttcccaga	6840
gacgcccagg	ccgaagttca	gatgactaac	tcaggggccc	agcttgcggg	cggctttcgt	6900
cacaggggtg	ggtcgcccgg	gcaggggtata	actcacctga	caatcagagg	gcgaggtatt	6960
cagctcaacg	acgagtcggg	gagctcctcg	ccttggtctcc	gtccggacgg	gacattttac	7020
atcggcggcg	ccggccgctc	ttcattcacg	cctcgtcagg	caatcctaac	tctgcagacc	7080
tcgtcctctg	agccgcgctc	tggaggcatt	ggaactctgc	aattttattga	ggagtttgtg	7140
ccatcgggtc	actttaaccc	cctctcgggg	cctcccggcc	actatccgga	tcaattttatt	7200
cctaactttg	acgcggtaaa	ggactcgggc	cacgggtacg	actgaatgtt	aagtggagag	7260
gcagagcaac	tgcgcctgaa	acacctgggt	cactgtcgcc	gccacaagtg	ccttgcccgc	7320
gactccggtg	agttttgcta	ccttgaattg	cccaggatc	atatcgaggg	cccggcgccac	7380
ggcgtccggc	ttaccgcccc	gggagagctt	gcccgtagcc	tgattcggga	gtttaccacg	7440
cgccccctgc	tagttgagcg	ggacagggga	cctgtgttcc	tactgtgat	ttgcaactgt	7500
cctaaccctt	gattacatca	agatctttgt	tgccatctct	gtgctgagta	taataaatac	7560
agaaattaaa	atatactggg	gtcctctatg	ccatcctgta	aacgccaccg	tcttccaccg	7620
cccaagcaaa	ccaaggcgaa	ccttacctgg	tacttttaac	atctctccct	ctgtgattta	7680
caacagtttc	aaccacagac	gagtgagctt	acgagagaac	ctctccgagc	tcagctactc	7740
catcagaaaa	aacaccaccc	tccttacctg	ccggggaacgt	acgagtgcgt	caccggccgc	7800
tgcaccacac	ctaccgcctg	accgtaaac	agactttttc	cggacagacc	tcaataactc	7860
tgtttaccag	aacaggaggt	gagcttagaa	aacctttagg	gtattaggcc	aaaggcgag	7920
ctactgtggg	gtttatgaac	aattcaagca	actctacggg	ctattctaat	tcagggtttct	7980
ctagataact	tcgtataatg	tatgctatac	gaagtatatg	tagaaatgga	cggaaattatt	8040

-108-

```

acagagcagc gcctgctaga aagacgcagg gcagcggccg agcaacagcg catgaatcaa 8100
gagctccaag acatgggttaa cttgcaccag tgcaaaaggg gtatcttttg tctggtaaag 8160
caggccaaag tcacctacga cagtaatacc accggacacc gccttagcta caagttgcca 8220
accaagcgct agaaattggg ggtcatgggt ggagaaaagc ccattaccat aactcagcac 8280
tcggtagaaa ccgaaggctg cattcactca ccttgtcaag gacctgagga tctctgcacc 8340
cttattaaga ccctgtgcgg tctcaaagat cttattccct ttaactaata aaaaaaata 8400
ataaagcatc acttacttaa aatcagttag caaattttctg tccagtttat tcagcagcac 8460
ctccttgccc tcctcccagc tctgggtattg cagcttctctc ctggctgcaa actttctcca 8520
caatctaaat ggaatgtcag tttcctcctg ttctgtcca tccgcaccca ctatcttcat 8580
gttggtgcag atgaagcgcg caagaccgtc tgaagatacc ttcaaccccg tgtatccata 8640
tgacacggaa accggtcctc caactgtgcc ttttcttact cctccctttg tatcccccac 8700
tgggtttcaa gagagtcccc ctgggggtact ctctttgcgc ctatccgaac ctctagttac 8760
ctccaatggc atgcttgcg ccaaaatggg caacggcctc tctctggacg aggccggcaa 8820
ccttacctcc caaaatgtaa ccactgtgag ccacctctc cccactaactg ggcctaactg 8880
aaacctggaa atatctgcac ccctcacagt taccctagaa ggcctaactg tggctgccgc 8940
cgcacctcta atggtcgcgg gcaacacact caccatgcaa tcacaggccc cgctaaccgt 9000
gcacgactcc aaacttagca ttgccacca aggaccctc acagtgtcag aaggaaagct 9060
agccctgcaa acatcaggcc ccctcaccac caccgatagc agtaccctta ctatcactgc 9120
ctcaccccct ctaactactg ccactggtag cttgggcatt gacttgaaag agccatttta 9180
tacacaaaat ggaaaactag gactaaagta cggggctcct ttgcatgtaa cagacgacct 9240
aaacactttg accgtagcaa ctgggtccagg tgtgactatt aataaactt ccttgcaaac 9300
taaagttact ggagccttgg gttttgattc acaaggcaat atgcaactta atgtagcagg 9360
aggactaagg attgattctc aaaacagacg ccttatactt gatgttagtt atccgtttga 9420
tgctcaaaac caactaatac taagactagg acagggccct ctttttataa actcagccca 9480
caacttggat attaactaca acaaaggcct ttacttgttt acagcttcaa acaattccaa 9540
aaagcttgag gttaacctaa gcaactgcaa ggggttgatg tttgacgcta cagccatagc 9600
cattaatgca ggagatgggc ttgaatttgg ttacactaat gcaccaaaca caaatccctc 9660
caaaacaaaa attggccatg gcctagaatt tgattcaaac aaggctatgg ttctaaact 9720
aggaactggc cttagttttg acagcacagg tgccattaca gtaggaaaca aaaataatga 9780
taagctaact ttgtggacca caccagctcc atctcctaac tgtagactaa atgcagagaa 9840
agatgctaaa ctcacttttg tcttaacaaa atgtggcagt caaatacttg ctacagtttc 9900
agttttggct gttaaaggca gtttggctcc aatatctgga acagttcaaa gtgctcatct 9960
tattataaga tttgacgaaa atggagtgtc actaaacaat tccttcctgg acccagaata 10020
ttggaacttt agaaatggag atcttactga aggcacagcc tatacaaacg ctggttgatt 10080
tatgcctaac ctatcagctt atccaaaatc tcacggtaaa actgccaaaa gtaacattgt 10140
cagtcaagtt tacttaaacg gagacaaaac taaacctgta acactaacca ttacactaaa 10200
cggtacacag gaaacaggag acacaactcc aagtgcatac tctatgtcat tttcatggga 10260
ctgggtctggc cacaactaca ttaatgaaat atttgccaca tcctcttaca ctttttcata 10320
cattgcccac gaataaagaa tcgttttgtt tatgtttcaa cgtgtttatt tttcaattgc 10380
agaaaatttc aagtcatttt tcattcagta gtatagcccc accaccacat agcttataca 10440
gatcaccgta ccttaatcaa actcacagaa ccctagtatt caacctgcca cctccctccc 10500
aacacacaga gtacacagtc ctttctcccc ggctggcctt aaaaagcatc atatcatggg 10560
taacagacat attcttaggt gttatattcc acacggtttc ctgtcgagcc aaacgctcat 10620
cagtgatatt aataaactcc ccgggcagct cacttaagtt catgtcgctg tccagctgct 10680
gagccacagg ctgctgtcca acttgcggtt gcttaacggg cggcgaaagg gaagtccacg 10740
cctacatggg ggtagagtca taatcgtgca tcaggatagg gcggtggtgc tgcagcagcg 10800
cgcgataaaa ctgctgccgc cgccgctccg tcctgca 10837

```

<210> 93
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> MunI TOP oligo

<400> 93
 aattgtgtta tgtttaaacg tgtttatttt tg

32

<210> 94
 <211> 32

-109-

<212> DNA
<213> Artificial Sequence

<220>
<223> MunI BOTTOM oligo

<400> 94
aattcaaaaa taaacacggt taaacataac ac

32

<210> 95
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer F37 5'SphI

<400> 95
taccaatggc atgctatccc tcaagg

26

<210> 96
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer F37 3'EcoRI

<400> 96
aaacacggga attcgtcttt cattc

25

<210> 97
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer F16 5'SphI

<400> 97
gccagcggca tgctccaact taaa

24

<210> 98
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer F16 3'MunI

<400> 98
tttatcaatt gtgttggtcag tcatcttc

28

<210> 99
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward Primer (TPL exon 3)

-110-

<400> 99
 ctcaacaatt gtggatccgt actcc 25
 <210> 100
 <211> 25
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Reverse Primer (TPL exon 3)
 <400> 100
 gtgctcagca gatcttgcca ctgtg 25
 <210> 101
 <211> 25
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Forward Primer (TPL exons 1/2)
 <400> 101
 ggcgcgttcg gatccactct cttcc 25
 <210> 102
 <211> 28
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Reverse Primer (TPL exons 1/2)
 <400> 102
 ctacatgcta ggcagatctc gttcggag 28
 <210> 103
 <211> 1240
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> TPL sequence with restriction sites
 <400> 103
 ggatccactc tcttcgcgat cgtgtgtctgc gagggccagc tgttgggggtg agtactccct 60
 ctgaaaagcg ggcattgactt ctgcgctaag attgtcagtt tccaaaaacg aggaggattt 120
 gatattcacc tggccgcgcg tgatgccttt gaggggtggcc gcatccatct ggtcagaaaa 180
 gacaatcttt ttgttgtcaa gcttggtggc aaacgacccg tagaggggcgt tggacagcaa 240
 cttggcgatg gagcgaggg tttgggtttt gtccgcgatcg gcgcgctcct tggccgcgat 300
 gtttagctgc acgtattcgc gcgcaacgca ccgccattcg ggaaagacgg tgggtgcgctc 360
 gtcgggcacc aggtgcacgc gccaacccgc gttgtgcagg gtgacaaggt caacgctggg 420
 ggctacctct ccgcgtaggc gctcgttggg ccagcagagg cggccgccct tgcgcgagca 480
 gaatggcggt aggggggtcta gctgcgtctc gtccggggggg tctgcgtcca cggtaaagac 540
 cccgggcagc aggcgcgcgt cgaagtagtc tatcttgcat ccttgcaagt ctacgcctg 600
 ctgccatgcg cgggcggcaa gcgcgcgctc gtatgggttg agtgggggac cccatggcat 660
 ggggtgggtg agcgcggagg cgtacatgcc gcaaatgtcg taaacgtaga ggggctctct 720
 gattattcca agatatgtag ggtagcatct tccaccgcgg atgctggcgc gcacgtaatc 780
 gtatagttcg tgcgaggag cgaggaggtc gggaccgagg ttgctacggg cgggctgctc 840
 tgctcggaag actatctgcc tgaagatggc atgtgagttg gatgatatgg ttggacgctg 900
 gaagacgttg aagctggcgt ctgtgagacc taccgcgtca cgcacgaagg aggcgtagga 960

-111-

```

gtcgcgcagc ttgttgacca gctcggcggt gacctgcacg tctagggcgc agtagtccag 1020
ggtttccttg atgatgtcat acttatcctg tccctttttt ttccacagct cgcggttgag 1080
gacaaactct tcgcggtctt tccagttact ttggatcgga aaccgcgcgc cctccgaacg 1140
agatccgtac tccgcgcgcg agggacctga gcgagtcgcg atcgaccgga tcggaaaacc 1200
tctcgagaaa ggcgtctaac cagtcacagt cgcaagatct          1240

```

<210> 104
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pBHG10 forward primer

<400> 104
 tgtacaccgg atccggcgca cacc 24

<210> 105
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pBHG10 reverse primer

<400> 105
 cacaacgagc tcaattaatt aattgccaca tcctc 35

<210> 106
 <211> 32480
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ad5. β gal. Δ F

<400> 106
 catcatcaat aatatacctt attttggatt gaagccaata tgataatgag ggggtggagt 60
 ttgtgacgtg gcgcggggcg tgggaacggg gcgggtgacg tagtagtgtg gcggaagtgt 120
 gatgttgcaa gtgtggcgga acacatgtaa gcgacggatg tggcaaaagt gacgtttttg 180
 gtgtgcgcgc gtgtacacag gaagtgaaca ttttcgcgcg gttttaggcg gatgtttag 240
 taaatttggg cgtaaccgag taagatttgg ccattttcgc gggaaaactg aataagagga 300
 agtgaaaatc gaataatttt gtgttactca tagcgcgtaa tctctagcat cgatgtcgac 360
 aagcttgaat tcgattaatg tgagttagct cactcattag gcaccccagg ctttacactt 420
 tatgcttccg gctcgtatgt tgtgtggaat tgtgagcggg taacaatttc acacaggaaa 480
 cagctatgac catgattacg aattcggcgc agcaccatgg cctgaaataa cctctgaaag 540
 aggaacttgg ttaggtacct tctgaggcgg aaagaaccag ctgtggaatg tgtgtcagtt 600
 aggggtgtgga aagtccccag gctccccagc aggcagaagt atgcaaagca tgcattctcaa 660
 ttagtcagca accaggtgtg gaaagtcccc aggtccccca gcaggcagaa gtatgcaaag 720
 ctatgcattc caattagtcag caaccatagt cccgccccta actccgccc accccccct 780
 aactccgccc agttccgccc attctccgcc ccatggctga ctaatttttt ttatttatgc 840
 agaggccgag gccgcctcgg cctctgagct attccagaag tagtgaggag gcttttttgg 900
 aggcctaggc ttttgcaaaa agcttgggat ctctataatc tcgcgcaacc tattttcccc 960
 tcgaacactt ttttaagcgt agataaacag gctgggacac ttcacatgag cgaaaaaatc 1020
 atcgctacct gggacatggt tattgcccga agccgtggcg gtctggtacc ggtgggtgaa 1140
 gaccagaaac agcacctcga actgagccgc gatattgccc agcgtttcaa cgcgctgtat 1200
 ggcgagatcg atcccgtcgt tttaacaagt cgtgactggg aaaaccctgg cgttacccaa 1260
 cttaatcgcc ttgcagcaca tcccccttcc gccagctggc gtaatatgca agaggcccg 1320
 accgatcgcc cttcccaaca gttgcgcagc ctgaatggcg aatggcgctt tgccgtggtt 1380
 ccggcaccag aagcgggtgc ggaaagctgg ctggagtgcg atcttcctga ggccgatact 1440

-112-

gtcgtcgtcc	cctcaaaactg	gcagatgcac	ggttacgatg	cgcccatcta	caccaacgta	1500
acctatccca	ttacgggtcaa	tccgcggttt	gttcccacgg	agaatccgac	gggttggttac	1560
tgcgtcacat	ttaatgttga	tgaaagctgg	ctacaggaag	gccagacgcg	aattattttt	1620
gatggcggtta	actcggcggtt	tcactctgtgg	tgcaacgggc	gctgggtcgg	ttacggccag	1680
gacagtcgtt	tgccgtctga	atthgacctg	agcgcattht	tacgcgccgg	agaaaaccgc	1740
ctcgcgggtga	tggtgctgcg	ttggagtgac	ggcagttatc	tggaagatca	ggatatgtgg	1800
cggatgagcg	gcattttccg	tgacgtctcg	ttgctgcata	aaccgactac	acaaatcagc	1860
gattttccatg	ttgccactcg	ctttaatgat	gattttcagcc	gcgctgtact	ggaggctgaa	1920
gttcagatgt	gcggcgagtt	gogtgactac	ctacgggtaa	cagttttcttt	atggcagggt	1980
gaaacgcagg	tcgccagcgg	caccgcgcct	ttcggcggtg	aaattatcga	tgagcgtggt	2040
ggttatgcgg	atcgcgtcac	actacgtctg	aacgtcgaaa	acccgaaact	gtggagcgcc	2100
gaaatcccg	atctctatcg	tgccgtggtt	gaactgcaca	ccgccgacgg	cacgctgatt	2160
gaagcagaag	cctgcgatgt	cgggtttccgc	gaggtgcgga	ttgaaaatgg	tctgctgctg	2220
ctgaacggca	agccgttgct	gattcagagg	gttaaccgtc	acgagcatca	tcctctgcat	2280
ggtcagggtca	tggtatgagca	gacgatgggtg	caggatatcc	tgctgatgaa	gcagaacaac	2340
tttaacggcg	tgccgtgttc	gcattatccg	aaccatccgc	tgtggtacac	gctgtgcgac	2400
cgtacggcc	tgatgtgggt	ggatgaagcc	aatattgaaa	cccacggcat	ggtgccaatg	2460
aatcgtctga	ccgatgatcc	gcgctggcta	ccggcgatga	gcgaacgcgt	aacgcgaatg	2520
gtgcagcgcg	atcgtaatca	cccagagtgtg	atcatctggt	cgctggggaa	tgaatcaggc	2580
cacggcgcta	atcacgacgc	gctgtatcgc	tggtacaaat	ctgtcgatcc	ttcccgcccg	2640
gtgcagtatg	aaggcgccgg	agccgacacc	acggccaccg	atattatttg	cccgatgtac	2700
gcgcgcgtgg	atgaagacca	gccccttcccg	gctgtgccga	aatgggtccat	caaaaaatgg	2760
ctttcgctac	ctggagagac	gcgcccgcgtg	atcctttgctg	aatacgccc	cgcgatgggt	2820
aacagtcttg	gcggtttcgc	taaatactgg	caggcggtttc	gtcagtatcc	ccgtttacag	2880
ggcggcttcg	tctgggactg	ggtggatcag	tcgctgatta	aatatgatga	aaacggcaac	2940
ccgtggtcgg	cttacggcgg	tgatttttggc	gatacgcga	acgatcgcca	gttctgtatg	3000
aacggtctgg	tctttgcgga	ccgcacgcgc	catccagcgc	tgacggaagc	aaaacaccag	3060
cagcagtttt	tccagttccg	tttatccggg	caaaccatcg	aagtgaccag	cgaataacctg	3120
ttccgtcata	gcgataacga	gctcctgcac	tggtatgggtg	cgctggatgg	taagccgctg	3180
gcaagcggtg	aagtgcctct	ggatgtcgct	ccacaaggta	aacagttgat	tgaactgcct	3240
gaactaccgc	agccggagag	cgccgggcaa	ctctggtcca	cagtacgcgt	agtgcaaccg	3300
aacgcgaccg	catggtcaga	agccgggcac	atcagcgctt	ggcagcagtg	gcgtctggcg	3360
gaaaacctca	gtgtgacgct	ccccgcgcgc	tcccacgcca	tcccgcatct	gaccaccagc	3420
gaaatggatt	tttgcatcga	gctgggtaat	aagcgttggc	aatttaaccg	ccagtcaggc	3480
tttctttcac	agatgtggat	tggcgataaa	aaacaactgc	tgacgccgct	gcgcgatcag	3540
tttccaccgtg	caccgctgga	taacgacatt	ggcgtaagt	aagcgaccgc	cattgacctt	3600
aacgcctggg	tcgaacgctg	gaaggcgggc	ggccattacc	aggccgaagc	agcgttgttg	3660
cagtgcacgg	cagatacact	tgctgatgcg	gtgctgatta	cgaccgctca	cgctggcag	3720
catcagggga	aaaccttatt	tatcagccgg	aaaacctacc	ggattgatgg	tagtgggtcaa	3780
atggcgatga	ccgttgatgt	tgaagtggcg	agcgatacac	cgcatccggc	gcggattggc	3840
ctgaactgcc	agctggcgca	ggtagcagag	cggttaaaact	ggctcggatt	agggcgcaa	3900
gaaaactatc	ccgaccgcct	tactgcgcgc	tgttttgacc	gctgggatct	gccattgtca	3960
gacatgtata	ccccgtacgt	cttcccagag	gaaaacgggtc	tgccgtgcgg	gacgcgcgaa	4020
ttgaattatg	gcccacacca	gtggcgcggc	gacttccagt	tcaacatcag	ccgctacagt	4080
caacagcaac	tgatggaaac	cagccatcgc	catctgctgc	acgcggaaga	aggcacatgg	4140
ctgaatatcg	acggttttcca	tatggggatt	gggtggcgacg	actcctggag	cccgtcagta	4200
tcggcggaat	tccagctgag	cgccgggtcg	taccattacc	agttgggtctg	gtgtcaaaaa	4260
taataataaa	cgggcaggcc	atgtctgccc	gtattttcgcg	taaggaaatc	cattatgtac	4320
tatttaaaaa	acacaaactt	ttggatgttc	ggtttattct	ttttctttta	cttttttatc	4380
atgggagcct	acttcccgtt	tttcccgtat	tggttgcacg	acatcaacca	tatcagcaaa	4440
agtgatacgg	gtattattht	tgccgctatt	tctctgttct	cgctattatt	ccaaccgctg	4500
tttgggtctgc	tttctgacaa	actcggaact	tgtttattgc	agcttataat	ggttacaaat	4560
aaagcaatag	catcacaaat	ttcacaaata	aagcattttt	ttcactgcat	tctagttgtg	4620
gtttgtccaa	actcatcaat	gtatcttatt	atgtctggat	ccagatctgg	gcgtggctta	4680
aggggtgggaa	agaatatata	aggtgggggt	cttatgtagt	tttgtatctg	ttttgcagca	4740
gccgccgccc	ccatgagcac	caactcgttt	gatggaagca	ttgtgagctc	atatttgaca	4800
acgcgcgatgc	ccccatgggc	cgggggtgct	cagaatgtga	tggtgtccag	cattgatggg	4860
cgccccgtcc	tgcccgcmaa	ctctactacc	ttgacctacg	agaccgtgtc	tggaacgcgc	4920
ttggagactg	cagcctccgc	cgcgcttcca	ggcgtgcag	ccaccgccc	cgggattgtg	4980
actgactttg	ctttcctgag	cccgtttgca	agcagtgacg	cttcccgttc	atccgcccgc	5040
gatgacaagt	tgacggctct	tttggcacaa	ttggattctt	tgaccgggga	acttaatgtc	5100

-113-

gtttctcagc	agctgttgga	tctgcgccag	caggttttctg	ccctgaaggc	ttcctcccct	5160
cccaatgcgg	tttaaaacat	aaataaaaaa	ccagactctg	tttggatttg	gatcaagcaa	5220
gtgtcttgct	gtcttttattt	aggggttttg	cgcgcgcggg	aggcccgga	ccagcgggtct	5280
cggtcgttga	gggtcctgtg	tattttttcc	aggacgtggg	aaagggtgact	ctggatgttc	5340
agatacatgg	gcataagccc	gtctctgggg	tggaggtagc	accactgcag	agcttcatgc	5400
tgcgggggtgg	tgtttagat	gatccagtcg	tagcaggagc	gctgggcgtg	gtgcctaaaa	5460
atgtctttca	gtagcaagct	gattgccagg	ggcaggccct	tgggtgtaagt	gtttacaaaag	5520
cggttaagct	gggatgggtg	catacgtggg	gatatgagat	gcctcttgga	ctgtattttt	5580
aggttggcta	tgttcccagc	catatccctc	cggggattca	tgttgtgcag	aaccaccagc	5640
acagtgtatc	cgggtgcactt	gggaaatttg	tcatgtagct	tagaaggaaa	tgcgtggaag	5700
aacttgagga	cgcccttgtg	acctccaaga	ttttccatgc	attcgtccat	aatgatggca	5760
atggggccac	ggggcgccgc	ctgggcgaag	atatttctgg	gatcactaac	gtcatagttg	5820
tgttccagga	tgagatcgtc	ataggccatt	tttacaagc	gcgggcggag	ggtgccagac	5880
tgcgggtataa	tggttccatc	cggcccaggg	gcgtagttac	cctcacagat	ttgcatttcc	5940
cacgctttga	gttcagatgg	ggggatcatg	tctacctgcg	gggcgatgaa	gaaaacgggt	6000
tccggggtag	gggagatcag	ctgggaagaa	agcaggttcc	tgagcagctg	cgacttaccg	6060
cagccgggtg	gcccgtaaat	cacacctatt	accgggtgca	actggtagtt	aagagagctg	6120
cagctgccgt	catccctgag	caggggggccc	acttcgttaa	gcctgtccct	gactcgcatg	6180
ttttccctga	ccaaatccgc	cagaaggcgc	tgcgcgccca	gcgatagcag	ttcttgcaag	6240
gaagcaaagt	ttttcaacgg	tttgagaccg	tccgcgtag	gcctgctttt	gagcgtttga	6300
ccaagcagtt	ccaggcgggtc	ccacagctcg	gtcacctgct	ctacggcatc	tcgatccagc	6360
atatctcttc	gtttcgcggg	ttggggcgcc	tttcgctgta	cggcagtagt	cgggtgctctg	6420
ccagacggggc	cagggtcatg	tctttccacg	ggcgcagggt	cctcgtcagc	gtagtctggg	6480
tcacgggtgaa	ggggtgcgct	ccgggctgcg	cgctggccag	ggtgcgcttg	aggctgggtcc	6540
tgctgggtgct	gaagcgctgc	cggctcttcgc	cctgcgcgctc	ggccaggtag	catttgacca	6600
tggtgtcata	gtccagcccc	tccgcggcgt	ggcccttggc	gcgcagcttg	cccttgagg	6660
aggcgccgca	cgaggggcag	tgcagacttt	tgagggcgta	gagcttgggc	gcgagaaata	6720
ccgattccgg	ggagtaggca	tccgcgcgcg	aggcccgca	gacgggtctcg	cattccacga	6780
gccagggtgag	ctctggccgt	tccgggtcaa	aaaccagggt	tcccccatgc	tttttgatgc	6840
gtttcttacc	tctggtttcc	atgagccggg	gtccacgctc	ggtgacgaaa	aggctgtccg	6900
tgtccccgta	tacagacttg	agaggcctgt	cctcgagcgg	tgttccgcgg	tcctcctcgt	6960
atagaaatc	ggaccactct	gagacaaagg	ctcgcgtcca	ggccagcacg	aaggaggcta	7020
agtgggaggg	gtagcggctc	ttgtccacta	gggggtccac	tcgctccagg	gtgtgaagac	7080
acatgtcgcc	ctcttcggca	tcaaggaagg	tgattgggtt	gtaggtgtag	gccacgtgac	7140
cgggtgttcc	tgaagggggg	ctataaaagg	gggtgggggc	gcgttcgtcc	tcactctctt	7200
ccgcacgcgt	gtctgcgagg	gccagctggt	ggggtgagta	ctccctctga	aaagcgggca	7260
tgaactctgc	gctaagattg	tcagtttcca	aaaacgagga	ggatttgata	ttcacctggc	7320
ccgcgggtgat	gcctttgagg	gtggccgcat	ccatctgggtc	agaaaagaca	atctttttgt	7380
tgtcaagctt	ggtggcaaac	gacccgtaga	gggcgttgga	cagcaacttg	gcgatggagc	7440
gcagggtttg	gtttttgtcg	cgatcggcgc	gctccttggc	cgcgatgttt	agctgcacgt	7500
attcgcgcgc	aaccgcacgc	cattcgggaa	agacgggtgg	gcgctcgtcg	ggcaccaggt	7560
gcacgcgcca	accgcgggtg	tgcagggtga	caagggtcaac	gctgggtggct	acctctccgc	7620
gtaggcgctc	gttgggtccag	cagaggcggc	cgcccttgcg	cgagcagaat	ggcggtaggg	7680
ggtctagctg	cgtctcgtcc	gggggggtctg	cgtccacggg	aaagaccccg	ggcagcaggc	7740
gcgcgtcgaa	gtagtctatc	ttgcacacct	gcaagtctag	cgctgctgc	catgcgcggg	7800
cggcaagcgc	gcgctcgat	gggttgagtg	ggggacccca	tggcatgggg	tgggtgagcg	7860
cggaggcgta	catgccgcaa	atgtcgtaaa	cgtagagggg	ctctctgagt	attccaagat	7920
atgtagggtg	gcctcttcca	ccgcggatgc	tggcgcgcac	gtaatcgat	agttcgtgcg	7980
agggagcgag	gaggtcggga	ccgaggttgc	tacgggcggg	ctgctctgct	cgggaagacta	8040
ctgcctgaa	gatggcatgt	gagttggatg	atatggttgg	acgctggaag	acgttgaagc	8100
tggcgtctgt	gagacctacc	gcgtcacgca	cgaaggaggc	gtaggagtcg	cgcagcttgt	8160
tgaccagctc	ggcggtgacc	tgcacgtcta	gggcgcagta	gtccagggtt	tccttgatga	8220
tgtcatactt	atcctgtccc	ttttttttcc	acagctcgcg	gttgaggaca	aactcttcgc	8280
ggtctttcca	gtactcttgg	atcgaaacc	cgtcggcctc	cgaacggtaa	gagcctagca	8340
tgtagaactg	gttgacggcc	tggtaggcgc	agcatccctt	ttctacgggt	agcgcgtatg	8400
cctgcgcggc	cttccggagc	gaggtgtggg	tgagcgcaaa	ggtgtccctg	accatgactt	8460
tgaggtagctg	gtatttgaag	tcagtgtcgt	cgcatccgoc	ctgctcccag	agcaaaaagt	8520
ccgtgcgctt	tttggaaacgc	ggatttggca	gggcgaaggt	gacatcggtg	aagagtatct	8580
ttccgcgcgc	aggcataaag	ttgcgtgtga	tgcggaaggg	tcccgccacc	tcggaacggc	8640
tgtaaatctac	ctgggcggcg	agcacgatct	cgtcaaagcc	gttgatgttg	tggcccacaa	8700
tgtaaagtcc	caagaagcgc	gggatgccct	tgatggaagg	caatttttta	agttcctcgt	8760

-114-

aggtgagctc	ttcaggggag	ctgagcccgt	gctctgaaag	ggcccagctc	gcaagatgag	8820
ggttgggaagc	gacgaatgag	ctccacaggt	cacggggccat	tagcattttgc	aggtgggtcgc	8880
gaaaggtcct	aaactggcga	cctatggcca	ttttttctgg	ggtgatgcag	tagaaggtaa	8940
gcgggtcttg	ttcccagcgg	tcccaccaa	ggttcgcggc	taggtctcgc	gcggcagtc	9000
ctagaggctc	atctccgcgc	aacttcatga	ccagcatgaa	gggcacgagc	tgcttcccaa	9060
aggcccccac	ccaagtatag	gtctctacat	cgtaggtgac	aaagagacgc	tcggtgcgag	9120
gatgcgagcc	gatcgggaag	aactggatct	cccgccacca	attggaggag	tggtatttga	9180
tgtggtgaaa	gtagaagtcc	ctgcgacggg	ccgaacactc	gtgctggctt	ttgtaaaaac	9240
gtgcgcagta	ctggcagcgg	tgcaacgggt	gtacatcctg	cacgaggttg	acctgacgac	9300
cgcgcacaag	gaagcagagt	gggaatttga	gcccctcgcc	tggcgggttt	ggctgggtgt	9360
cttctacttc	ggctgcttgt	ccttgaccgt	ctggctgctc	gaggggagtt	acggtggatc	9420
ggaccaccac	gccgcgcgag	cccaaagtcc	agatgtccgc	gcgcggcggt	cggagcttga	9480
tgacaacatc	gcgcagatgg	gagctgtcca	tggtctggag	ctcccgcggc	gtcaggtcag	9540
gcgggagctc	ctgcaggttt	acctcgcata	gacgggtcag	ggcgcgggct	agatccaggt	9600
gatacctaata	ttccaggggc	tggttggtgg	cggcgtcgat	ggcttgcaag	aggccgcata	9660
cccgcggcgc	gactacggta	ccgcgcggcg	ggcggtgggc	cgcgggggtg	tccttggtatg	9720
atgcatactaa	aagcggtgac	gcgggcgagc	cccgcgaggt	agggggggct	ccggaccgcg	9780
cgggagaggg	ggcaggggca	cgtcggcgcc	gcgcgcgggc	aggagctggt	gctgcgcgcg	9840
taggttgctg	gcgaacgcga	cgacgcggcg	gttgatctcc	tgaatctggc	gcctctgcgt	9900
gaagacgacg	ggcccgggtga	gcttgagcct	gaaagagagt	tcgacagaat	caatttcggt	9960
gtcgttgacg	gcggcctggc	gcaaaatctc	ctgcacgtct	cctgagttgt	cttgataggc	10020
gatctcggcc	atgaactgct	cgatctcttc	ctcctggaga	tctccgcgtc	cggctcgctc	10080
cacggtggcg	gcgaggtcgt	tggaatgctg	ggccatgagc	tgcgagaagg	cgttgaggcc	10140
tccctcgttc	cagacgcggc	tgtagaccac	gcccccttcg	gcacgcggg	cgcgcatgac	10200
cacctgcgcg	agattgagct	ccacgtgccc	ggcgaagacg	gcgtagtttc	gcaggcgctg	10260
aaagaggtag	ttgaggggtg	tggcgggtgtg	ttctgccacg	aagaagtaca	taaccacagc	10320
tcgcaacgtg	gattcggtga	tatcccccaa	ggcctcaagg	cgctccatgg	cctcgtagaa	10380
gtccacggcg	aagttgaaaa	actgggagtt	gcgcgccgac	acggttaact	cctcctccag	10440
aagacggatg	agctcggcga	cagtgtcgcg	cacctcgcg	tcaaaggcta	caggggcctc	10500
ttcttcttct	tcaatctcct	cttcataaag	ggcctccct	tcttcttctt	ctggcggcgg	10560
tgggggaggg	gggacacggc	ggcgacgcag	gcgcaccggg	aggcggtcga	caaagcgctc	10620
gatcatctcc	gcgcggcgac	ggcgcatggt	ctcgggtgacg	gcgcggccgt	tctcgcgggg	10680
gcgcagttgg	aagacgcgcg	ccgtcatgtc	ccggttatgg	gttggcgggg	ggctgccatg	10740
cggcagggat	acggcgctaa	cgatgcatac	caacaattgt	tgtgtaggta	ctccgcgcgc	10800
gagggacctg	agcgagtccg	catcgaccgc	atcggaaaaa	ctctcgagaa	aggcgtctaa	10860
ccagtacacg	tcgcaaggta	ggctgagcac	cgtggcggcg	ggcagcgggc	ggcggtcggg	10920
gttgtttctg	gcggaggtgc	tgctgatgat	gtaattaaag	taggcgggtc	tgagacggcg	10980
gatggtcgac	agaagcacca	tgctcttggg	tccggcctgc	tgaatgcgca	ggcggtcggc	11040
catgccccag	gcttcgtttt	gacatcggcg	caggtctttg	tagtagtctt	gcatagacct	11100
ttctacggcg	acttctctt	ctccttctct	ttgtcctgca	tctcttgcat	ctatcgctga	11160
ggcgccggcg	gagtttggcc	gtaggtggcg	ccctcttctc	cccatgcgtg	tgaccctgaa	11220
gcccctcatc	ggctgaagca	gggctaggtc	ggcgacaacg	cgctcggcta	atatggcctg	11280
ctgcacctgc	gtgagggtag	actggaagtc	atccatgtcc	acaaagcggt	ggtatgcgcc	11340
cgtgttgatg	gtgtaagtgc	agttggccat	aacggaccag	ttaacggtct	ggtgaccgcg	11400
ctgcgagagc	tcggtgtacc	tgagacgcga	gtaagccctc	gagtcaaata	cgtagtcggt	11460
gcaagtccgc	accaggtact	ggtatcccac	caaaaagtgc	ggcggcggct	ggcggtagag	11520
gggcccagcgt	agggtggccg	gggctccggg	ggcgagatct	tccaacataa	ggcgatgata	11580
tccgtagatg	tacctggaca	tccaggtgat	gcccggcgcg	gtggtggagg	gcgcgggaaa	11640
gtcgcggacg	cggttccaga	tggtgcgcag	cggcaaaaaa	tgctccatgg	tcgggacgct	11700
ctggccgggtc	aggcgcgcgc	aatcgttgac	ggtctagacc	gtgcaaaaag	agagcctgta	11760
agcgggcact	cttcggtggt	ctggtggata	aattcgcaag	ggtatcatgg	cggacgaccg	11820
gggttcgagc	cccgtatccg	gccgtccgcc	gtgatccatg	cggttaccgc	ccgcgtgtcg	11880
aaaccagggtg	tgcgacgtca	gacaacgggg	gagtgtctct	tttggcttcc	ttccaggcgc	11940
ggcggctgct	gcgctagctt	ttttggccac	tggcgcgcgc	cagcgtaagc	ggttaggctg	12000
gaaagcgaaa	gcattaagtg	gctcgtctcc	tgtagccgga	gggttatttt	ccaagggttg	12060
agtgcgcggga	cccccggttc	gagtcctcga	ccggccggac	tgccggcgaac	gggggtttgc	12120
ctccccgtca	tgcaagaccc	cgcttgcaaa	ttcctccgga	aacagggacg	agcccttttt	12180
ttgctttttcc	cagatgcata	cggtgctgcg	gcagatgcgc	ccccctctct	agcagcggca	12240
agagcaagag	cagcgaggca	catgcaggga	acctctccct	cctcctaccg	cgtcaggagg	12300
ggcgacatacc	gcgggtgacg	cggcagcaga	tggtgattac	gaacccccgc	ggcgccgggc	12360
ccggcactac	ctggacttgg	aggagggcga	gggcctggcg	cggctaggag	cgccctctcc	12420

-115-

tgagcgggtac	ccaaggggtgc	agctgaagcgy	tgatacgcgt	gagggcgtacg	tgccgcgggca	12480
gaacctgttt	cgcgaccgcg	agggagagga	gcccagaggag	atgcgggatc	gaaagttcca	12540
cgacgggcgc	gagctgcggc	atggcctgaa	tcgcgagcgg	ttgctgcgcg	aggaggactt	12600
tgagcccgac	gcgcgaaccg	ggattagtc	cgcgcgcgca	cacgtggcg	ccgccgacct	12660
ggtaaccgca	tacgagcaga	cggtgaacca	ggagattaac	tttcaaaaaa	gctttaacaa	12720
ccacgtgctg	acgcttgtgg	cgcgcgagga	ggtggctata	ggactgatgc	atctgtggga	12780
ctttgtaagc	gcgctggagc	aaaacccaaa	tagcaagccg	ctcatggcg	agctgttcct	12840
tatagtgcag	cacagcaggg	acaacgaggg	attcagggat	gcgctgctaa	acatagtaga	12900
gcccaggggc	cgctggctgc	tcgatttgat	aaacatcctg	cagagcatag	tggtgcagga	12960
gcgcagcttg	agcctggctg	acaaggtggc	cgccatcaac	tattccatgc	ttagcctggg	13020
caagttttac	gcccgcgaaga	tataccatac	cccttacgct	cccatagaca	aggaggtaaa	13080
gatcgagggg	ttctacatgc	gcatggcgct	gaaggtgctt	accttgagcg	acgacctggg	13140
cgtttatcgc	aacgagcgca	tcacaaaggc	cgtgagcgtg	agccggcgcg	gcgagctcag	13200
cgaccgcgag	ctgatgcaca	gcctgcaaag	ggccctggct	ggcacgggca	gcggcgatag	13260
agaggccgag	tcctactttg	acgcggggcg	tgacctgcgc	tgggcccca	gccgacgcgc	13320
cctggaggca	gctggggccg	gacctgggct	ggcggtggca	ccgcgcgcgc	ctggcaacgt	13380
cgcgcgctg	gaggaatatg	acgaggacga	tgagtacgag	ccagaggacg	gcgagtagta	13440
agcggtagtg	tttctgatca	gatgatgcaa	gacgcaacgg	acccggcggt	gcgggcggcg	13500
ctgcagagcc	agccgtccgg	ccttaactcc	acggacgact	ggcgccagg	catggaccgc	13560
atcatgtcgc	tgactgcgcg	caatcctgac	gcgttccggc	agcagccgca	ggccaaccgg	13620
ctctccgcaa	ttctggaagc	ggtgggtccc	gcgcgcgcaa	accccacgca	cgagaagggtg	13680
ctggcgatgc	taaacgcgct	ggcgaaaaac	agggccatcc	ggcccgacga	ggccggcctg	13740
gtctacgacg	cgctgcttca	gcgcgtggct	cgttacaaca	gcggcaacgt	gcagaccaac	13800
ctggaccggc	tggtggggga	tgtgcgcgag	gcgtggcg	agcgtgagcg	cgcgacgag	13860
cagggcaacc	tggtgctccat	ggttgacta	aacgccttcc	tgagtacaca	gcccgcaca	13920
gtgcccggg	gacaggagga	ctacaccaac	tttgtgagcg	cactgcccgt	aatgggtgact	13980
gagacaccgc	aaagttaggt	gtaccagtct	gggcccagact	attttttcca	gaccagtaga	14040
caaggcctgc	agaccgtaaa	cctgagccag	gctttcaaaa	acttgaggg	gctgtggggg	14100
gtgcgggctc	ccacaggcga	ccgcgcgacc	gtgtctagct	tgctgacgcc	caactcgcgc	14160
ctgttgctgc	tgctaatagc	gccccttcacg	gacagtggca	gcgtgtccc	ggacacatac	14220
ctaggtcact	tgttgacact	gtaccgcgag	gccataggtc	aggcgcatgt	ggacgagcat	14280
actttccagg	agattacaag	tgtagccgc	cgctggggc	aggaggacac	gggcagcctg	14340
gaggcaacc	taaactacct	gctgaccaac	cgccggcaga	agatcccctc	gctgcacagt	14400
ttaaacagcg	aggaggagcg	catttttgcgc	tacgtgcagc	agagcgtgag	ccttaacctg	14460
atgcgcgacg	gggtaacgcc	cagcgtggcg	ctggacatga	ccgcgcgcaa	catggaaccg	14520
ggcatgtatg	cctcaaaccg	gccgtttatc	aaccgcctaa	tggaactact	gcactcgcgc	14580
gccgccgtga	accccgagta	tttaccat	gccatcttga	acccgcaactg	gctaccgccc	14640
cctggtttct	acaccggggg	attcgagggtg	cccgagggtg	acgatggatt	cctctggggac	14700
gacatagacg	acagcgtggt	ttccccgcaa	ccgcgagacc	tgctagagtt	gcaacagcgc	14760
gagcaggcag	aggcgggcgt	gcgaaaggaa	agcttccgca	ggccaagcag	cttgtccgat	14820
ctaggcgctg	cggcccccgc	gtcagatgct	agttagcccat	ttccaagctt	gataggtct	14880
cttaccagca	ctcgcaccac	ccgcgcgcgc	ctgctggcg	aggaggagta	cctaaacaac	14940
tcgctgctgc	agccgcagcg	cgaaaaaaac	ctgcctccgg	catttcccaa	caacgggata	15000
gagagcctag	tggacaagat	gagtagatgg	aagacgtacg	cgcaggagca	cagggacgtg	15060
ccaggccccg	gcccgcacc	ccgtcgtcaa	aggcacgacc	gtcagcgggg	tctgggtgtg	15120
gaggacgatg	actcggcaga	cgacagcagc	gtcctggatt	tgggaggagg	tggcaaccgc	15180
tttgccgacc	ttcgccccag	gctggggaga	atgtttttaa	aaaaaaaaag	catgatgcaa	15240
aataaaaaaac	tcaccaaggc	catggcaccg	agcgttgggt	ttcttgtatt	ccccttagta	15300
tgcggcgcg	ggcgatgtat	gaggaagggt	ctcctccctc	ctacgagagt	gtggtagagc	15360
cggcgccag	cgccggcg	ctgggtctc	ccttcgatgc	tcccctggac	ccgcgctttg	15420
tgccctccgc	gtacctgcg	cctaccgggg	ggagaaacag	catccgttac	tctgattggg	15480
caccctatt	cgacaccacc	cgtgtgtacc	tggtggacaa	caagtcaacg	gatgtggcat	15540
ccctgaacta	ccagaacgac	cacagcaact	ttctgaccac	ggtcattcaa	aacaatgact	15600
acagcccggg	ggaggcaagc	acacagacca	tcaactctga	cgaccggctg	cactggggcg	15660
cgacacctgaa	aaccatcctg	cataccaaca	tgccaaatgt	gaacgagttc	atgtttacca	15720
ataagtttaa	ggcgcggtg	atggtgtcgc	gcttgccctac	taaggacaat	caggtggagc	15780
tgaaatacga	gtgggtggag	ttcacgctgc	ccgagggcaa	ctactccgag	accatgacca	15840
tagaccttat	gaacaacgcg	atcgtggagc	actacttgaa	agtgggcaga	cagaacgggg	15900
ttctggaaag	cgacatcggg	gtaaaagtgt	acacccgcaa	ccttcagactg	gggtttgacc	15960
ccgtcactgg	cttgtcatg	cctgggttat	atataaacga	agccttccat	ccagacatca	16020
ttttgctgcc	aggatgcggg	gtggacttca	cccacagccg	cctgagcaac	ttgttgggca	16080

-116-

tccgcaagcg	gcaacccttc	caggagggct	ttaggatcac	ctacgatgat	ctggaggggtg	16140
gtaacattcc	cgcactgttg	gatgtggacg	cctaccaggc	gagcttgaaa	gatgacaccg	16200
aacagggcgg	gggtggcgca	ggcggcagca	acagcagtgg	cagcggcgcg	gaagagaaat	16260
ccaacgcggc	agccgcggca	atgcagccgg	tggaggacat	gaacgatcat	gccattcgcg	16320
gcgacacctt	tgccacacgg	gctgaggaga	agcgcgctga	ggccgaagca	gcggccgaag	16380
ctgccgcccc	cgctgcgcaa	cccagggctg	agaagcctca	gaagaaaccg	gtgatcaaac	16440
ccctgacaga	ggacagcaag	aaacgcagtt	acaacctaata	aagcaatgac	agcaccttca	16500
cccagtaccg	cagctgggtac	cttgcataca	actacggcga	ccctcagacc	ggaatccgct	16560
catggaccct	gctttgcact	cctgacgtaa	cctgcggctc	ggagcaggtc	tactggctcg	16620
tgccagacat	gatgcaagac	cccgtgacct	tccgctccac	gcgccagatc	agcaactttc	16680
cgggtgggtgg	cgccgagctg	ttgcccgtgc	actccaagag	cttctacaac	gaccaggccg	16740
tctactccca	actcttccca	cagttttacct	ctctgaccca	cgtgttcaat	cgctttcccg	16800
agaaccagat	tttggcgcg	ccgccagccc	ccaccatcac	caccgtcagt	gaaaacggtt	16860
ctgctctcac	agatcacggg	acgctaccgc	tgcgcaacag	catcggagga	gtccagcgag	16920
tgaccattac	tgacgccaga	cgccgcacct	gcccctacgt	ttacaaggcc	ctgggcatag	16980
tctcgccgcg	cgtcctatcg	agccgcacct	tttgagcaag	catgtccatc	cttatatcgc	17040
ccagcaataa	cacaggctgg	ggcctgcgct	tcccaagcaa	gatgtttggc	ggggccaaga	17100
agcgctccga	ccaacaccca	gtgcgcgtgc	gcgggcacta	ccgcgcgccc	tggggcgcg	17160
acaaaacgcg	ccgcactggg	cgcaccaccg	tcgatgacgc	catcgacgcg	gtgggtggagg	17220
aggcgcgcaa	ctacacgccc	acgcccgcac	cagtgtccac	agtggacgcg	gccattcaga	17280
ccgtgggtgcg	cggagcccg	cgctatgcta	aaatgaagag	acggcggagg	cgcgtagcac	17340
gtcgccaccg	ccgcccagcc	ggcactgcgc	cccaacgcg	ggcggcgcc	ctgcttaacc	17400
gcgcacgtcg	caccggccga	cgggcgccca	tgccggcgcg	tcgaaggctg	gcccgggta	17460
ttgtcactgt	gccccccagg	tccaggcgac	gagcgccgc	cgacgcagcc	gcggccatta	17520
gtgctatgac	tcagggtcgc	aggggcaacg	tgtattgggt	gcgcgactcg	gttagcgcc	17580
tgcgcggtgc	cgtgcgcacc	cgccccccgc	gcaactagat	tgcaagaaaa	aactacttag	17640
actcgtactg	ttgtatgtat	ccagcggcgg	cggcgcgcaa	cgaagctatg	tccaagcgca	17700
aaatcaaaga	agagatgctc	caggctcatcg	cgccggagat	ctatggcccc	ccgaagaagg	17760
aagagcagga	ttacaagccc	cgaaagctaa	agcgggtcaa	aaagaaaaag	aaagatgatg	17820
atgatgaact	tgacgacgag	gtggaactgc	tgacgcgtac	cgcgcccagg	cgacgggtac	17880
agtgcaaaag	tcgacgcgta	aaacgtgttt	tgcgacccgg	caccaccgta	gtcttttacgc	17940
ccggtgagcg	ctccaccgcg	acctacaagg	cggtgtatga	tgaggtgtac	ggcgacgagg	18000
acctgcttga	gcaggccaac	gagcgcctcg	gggagtttgc	ctacggaaag	cggcataaag	18060
acatgctggc	gttgccgctg	gacgagggca	acccaacacc	tagcctaagg	cccgtaacac	18120
tgacgacggt	gctgcccgcg	cttgcacccg	ccgaagaaaa	gcgcggccta	aagcgcgagt	18180
ctgggtgactt	ggcaccacc	gtgcagctga	tggtagccga	gcgccagcga	ctggaagatg	18240
tcttgaaaaa	aatgaccgtg	gaacctgggc	tggagccga	ggtccgcgtg	cggccaatca	18300
agcagggtgg	gccgggactg	ggcgtgcaga	ccgtggacgt	tcagataccc	actaccagta	18360
gcaccagtat	tgccaccgcc	acagagggca	tggagacaca	aacgtccccg	gttgccctcag	18420
cgggtggcgga	tgccgcgggtg	caggcgggtc	ctgcggccgc	gtccaagacc	tctacggagg	18480
tgcaaacgga	cccgctggatg	tttcgcgttt	cagcccccg	gcgcccgcgc	ggttcgagga	18540
agtacggcgc	cgccagcgcg	ctactgccc	aatatgccct	acatccttcc	attgcgccta	18600
cccccggtta	tcgtgggtac	acctaccgcc	ccagaagacg	agcaactacc	cgacgccgaa	18660
ccaccactgg	aaccgcgcgc	cgccgtcgcc	gtcgccagcc	cgtgctggcc	ccgatttccg	18720
tgcgcgagggt	ggctcgcgaa	ggaggcagga	ccctgggtgct	gccaacagcg	cgctaccacc	18780
ccagcatcgt	ttaaaagccg	gtctttgtgg	ttcttgacaga	tatggccctc	acctgccgcc	18840
tccgtttccc	ggtgccggga	ttccgaggaa	gaatgcaccg	taggaggggc	atggccggcc	18900
acggcctgac	gggcggcatg	cgtcgtgcgc	accaccggcg	gcggcgcgcg	tcgcaccgtc	18960
gcattcgcg	cggtatcctg	cccctcctta	ttccactgat	cgccgcggcg	attggcgccg	19020
tgcccggaat	tgcatccgtg	gccttgacgg	cgcagagaca	ctgattaaaa	acaagttgca	19080
tgtggaaaaa	tcaaaaataaa	aagtctggac	tctcacgctc	gcttgggtcct	gtaactatbt	19140
tgtagaatgg	aagacatcaa	ctttgcgtct	ctggccccgc	gacacggctc	gcgcccgttc	19200
atgggaaact	ggcaagatat	cggcaccagc	aatatgagcg	gtggcgccct	cagctggggc	19260
tcgctgtgga	gcggcattaa	aaatttcggg	tccaccgtta	agaactatgg	cagcaaggcc	19320
tggaaacagca	gcacaggcca	gatgctgagg	gataagttga	aagagcaaaa	tttccaacaa	19380
aagggtggtag	atggcctggc	ctctggcatt	agcggggtgg	tggacctggc	caaccaggca	19440
gtgcaaaaata	agattaacag	taagcttgat	ccccgcctc	ccgtagagga	gcctccaccg	19500
gccgtggaga	cagtgtctcc	agaggggcgt	ggcgaaaaagc	gtccgcgccc	cgacagggaa	19560
gaaactctgg	tgacgcgaat	agacgagcct	ccctcgtagc	aggaggcact	aaagcaaggc	19620
ctgcccacca	cccgctccat	cgcgcccctg	gctaccggag	tgctgggcca	gcacacacc	19680
gtaacgctgg	acctgcctcc	ccccgcgcac	accagcaga	aacctgtgct	gccaggcccc	19740

-117-

accgccgttg	ttgtaacccg	tcctagccgc	gcgtccctgc	gccgcgccgc	cagcggtccg	19800
cgatcggtgc	ggcccgtagc	cagtggcaac	tggcaaagca	cactgaacag	catcggtgggt	19860
ctgggggtgc	aatccctgaa	gcgcgcacga	tgccttctgaa	tagctaacgt	gtcgtatgtg	19920
tgtcatgtat	gcgtccatgt	cgccgccaga	ggagctgctg	agccgccgcg	cgcccgcctt	19980
ccaagatggc	tacccttctg	atgatgccgc	agtggctcta	catgcacatc	tcgggccagg	20040
acgcctcgga	gtacctgagc	cccgggctgg	tgcagtttgc	ccgcgccacc	gagacgtact	20100
tcagcctgaa	taacaagttt	agaaacccca	cggtaggcgc	tacgcacgac	gtgaccacag	20160
accggtccca	gcgttttgacg	ctgcggttca	tcctgtgga	ccgtgaggat	actgcgtact	20220
cgtacaaggc	gcggttcacc	ctagctgtgg	gtgataaaccg	tgtgctggac	atggcttcca	20280
cgtactttga	catccgcggc	gtgctggaca	ggggccctac	ttttaagccc	tactctggca	20340
ctgcctacaa	cgccctggct	cccaaggggtg	ccccaaatcc	ttgcgaatgg	gatgaagctg	20400
ctactgctct	tgaaaataaac	ctagaagaag	aggacgatga	caacgaagac	gaagtagacg	20460
agcaagctga	gcagcaaaaa	actcacgtat	ttgggcaggc	gccttattct	ggtataaata	20520
ttacaaagga	gggtattcaa	ataggtgtcg	aaggtcaaac	acctaaatat	gccgataaaa	20580
catttcaacc	tgaacctcaa	ataggagaat	ctcagtggta	cgaaactgaa	attaatcatg	20640
cagctgggag	agtccttaaa	aagactaccc	caatgaaacc	atgttacggt	tcatatgcaa	20700
aaccacaaaa	tgaaaatgga	gggcaaggca	ttcttgtaaa	gcaacaaaaat	ggaaagctag	20760
aaagtcaagt	ggaaatgcaa	tttttctcaa	ctactgaggc	gaccgcaggc	aatggtgata	20820
acttgactcc	taaagtggta	ttgtacagtg	aagatgtaga	tatagaaacc	ccagacactc	20880
atatttctta	catgcccact	attaagggaag	gtaactcacg	agaactaatg	ggccaacaat	20940
ctatgcccaa	caggcctaata	tacattgctt	ttagggacaa	ttttattggt	ctaattgtatt	21000
acaacagcac	gggttaatatg	gggttctctg	cgggccaagc	atcgcagttg	aatgctggtg	21060
tagatttgca	agacagaaac	acagagcttt	cataccagct	tttgcttgat	tccattggtg	21120
atagaaccag	gtacttttct	atgtggaatc	aggctgttga	cagctatgat	ccagatgtta	21180
gaattattga	aaatcatgga	actgaagatg	aacttccaaa	ttactgcttt	ccactgggag	21240
gtgtgattaa	tacagagact	cttaccaagg	taaaacctaa	aacaggtcag	gaaaatggat	21300
gggaaaaaga	tgctacagaa	ttttcagata	aaaatgaaat	aagagttgga	aataattttg	21360
ccatggaaat	caatctaaat	gccaacctgt	ggagaaaatt	cctgtactcc	aacatagcgc	21420
tgtatttgcc	cgacaagcta	aagtacagtc	cttccaacgt	aaaaatttct	gataacccaa	21480
acacctacga	ctacatgaac	aagcgagtgg	tggctcccgg	gttagtgagc	tgctacatta	21540
accttgagc	acgttggtcc	cttgactata	tggacaacgt	caaccatttt	aaccaccacc	21600
gcaatgctgg	cctgcgtac	cgctcaatgt	tgtggggcaa	tggctcgctat	gtgcccttcc	21660
acatccaggt	gcctcagaag	ttctttgcca	ttaaaaacct	ccttctcctg	ccgggctcat	21720
acacctacga	gtggaacttc	aggaaggatg	ttaacatggt	tctgcagagc	tccttaggaa	21780
atgacctaa	ggttgacgga	gccagcatta	agtttgatag	catttgccct	tacgccacct	21840
tcttcccat	ggcccacaac	cgcttgaggc	tcagctggcc	catgcttaga	aacgacacca	21900
acgaccagtc	ctttaacgac	tatctctccg	ccgccaacat	gctctaccct	ataccgcgca	21960
acgctaccaa	cgtgcccata	tccatcccct	ccgcgaactg	ggcggctttc	cgcggctggg	22020
ccttcacgcg	ccttaagact	aaggaaaccc	catcactggg	ctcgggctac	gacccttatt	22080
acacctactc	tggtctctata	ccctacctag	atggaacctt	ttacctcaac	cacaccttta	22140
agaaggtggc	cattacctttt	gactcttctg	tcagctggcc	tggcaatgac	cgctgcttta	22200
cccccaacga	gtttgaaatt	aagcgtcag	ttgacgggga	gggttacaac	gttgccagct	22260
gtaacatgac	caaagactgg	ttcctggtag	aaatgctagc	taactacaac	attggctacc	22320
agggttcta	tatcccagag	agctacaagg	accgcatgta	ctccttcttt	agaaaacttc	22380
agcccatgag	ccgtcagggtg	gtggatgata	ctaaatacaa	ggactaccaa	caggtgggca	22440
tcctacacca	acacaacaac	tctggatttg	ttggctacct	tgccccacc	atgcgcgaag	22500
gacaggccta	ccctgctaac	ttcccctatc	cgcttatagg	caagaccgca	gttgacagca	22560
ttaccagaa	aaagtttctt	tgcgatcgca	ccctttggcg	catcccatcc	tccagtaact	22620
ttatgtccat	gggcgcactc	acagacctgg	gccaaaacct	tctctacgcc	aactccgcct	22680
acgcgctaga	catgactttt	gaggtggatg	ccatggacga	gcccaccctt	ctttatgttt	22740
tgtttgaagt	ctttgacgtg	gtccgtgtgc	accggccgca	ccgcggcgctc	atcgaaaaccg	22800
tgtacctgcg	cacgcccttc	tcggccggca	acgccacaac	ataaagaagc	aagcaacatc	22860
aacaacagct	gccgccatgg	gctccagtag	gcaggaaactg	aaagccattg	tcaaagatct	22920
tggttggtgg	ccatattttt	tgggcacctc	tgacaagcgc	tttccaggct	ttgtttctctc	22980
acacaagctc	gcctgcgcca	tagtcaatac	ggccggtcgc	gagactgggg	gcgtacactg	23040
gatggccttt	gcctggaacc	cgcactcaaa	aacatgctac	ctctttgagc	cctttggctt	23100
ttctgaccag	cgactcaagc	aggtttacca	gtttgagtac	gagtcactcc	tgcgcgtag	23160
cgccattgct	tcttcccccg	accgctgtat	aacgctggaa	aagtccaccc	aaagcgtaca	23220
ggggcccaac	tcggcgccct	gtggactatt	ctgctgcacg	tttctccacg	cctttgccaa	23280
ctggcccaaa	actcccattg	atcacaaccc	caccatgaac	cttattaccg	gggtacccaa	23340
ctccatgctc	aacagtcccc	aggtagagcc	cacctgcgt	cgcaaccagg	aacagctcta	23400

-118-

cagcttcctg	gagcgccact	cgcctactt	ccgcagccac	agtgcgcaga	ttaggagcgc	23460
cacttctttt	tgtcacttga	aaaacatgta	aaaataatgt	actagagaca	ctttcaataa	23520
aggcaaatgc	ttttatttgt	acactctcgg	gtgattattt	acccccaccc	ttgccgtctg	23580
cgccgtttta	aaatcaaagg	ggttctgccc	cgcatcgcta	tgcgccactg	gcaggacac	23640
gttgcgatac	tggtgttttag	tgctccactt	aaactcaggc	acaaccatcc	gcggcagctc	23700
ggtgaagttt	tactccaca	ggctgcgcac	catcaccaac	gcgttttagca	ggtcgggcgc	23760
cgatatcttg	aagtgcgagt	tggggcctcc	gccctgcgcg	cgcgagttgc	gatacacagg	23820
gttgacgac	tggaacacta	tcagcgccgg	gtggtgcacg	ctggccagca	cgctcttgtc	23880
ggagatcaga	tccgcgtcca	ggctctccgc	gttgctcagg	gcgaacggag	tcaactttgg	23940
tagctgcctt	cccaaaaagg	gcgcgtgccc	aggctttgag	ttgcactcgc	accgtagtgg	24000
catcaaaagg	tgaccgtgcc	cggctctgggc	gttaggatac	agcgctgca	taaaagcctt	24060
gatctgctta	aaagccacct	gagcctttgc	gccttcagag	aagaacatgc	cgcaagactt	24120
gcgggaaaac	tgattggccg	gacaggccgc	gtcgtgcacg	cagcaccttg	cgtcgggtgt	24180
ggagatctgc	accacatttc	ggccccaccg	gttcttcaog	atcttggcct	tgctagactg	24240
ctccttcagc	gcgcgctgcc	cgttttcgc	cgtcacatcc	atttcaatca	cgctgctcctt	24300
atztatcata	atgcttccgt	gtagacactt	aagctgcgct	tcgatctcag	cgagcggtg	24360
cagccacaac	gcgcagcccg	tgggctcgtg	atgcttgtag	gtcacctctg	caaacgactg	24420
caggtacgcc	tgccaggaatc	gccccatcat	cgtcacaaag	gtcttgttgc	tggtgaaggt	24480
cagctgcaac	ccgcggtgct	cctcgttcag	ccaggtcttg	catacggccg	ccagagcttc	24540
cacttggtca	ggcagtagtt	tgaagttcgc	cttttagatcg	ttatccacgt	ggtacttgct	24600
catcagcgcg	cgcgagcctt	ccatgcctt	ctcccacgca	gacacgatcg	gcacactcag	24660
cggttccatc	accgtaattt	cactttccgc	ttoctggggc	tcttctctct	cctcttgctg	24720
cgccatacca	cgccgactg	ggctgctctt	attcagccgc	cgactgtgct	gcttacctcc	24780
tttgccatgc	ttgattagca	ccggtgggtt	gctgaaaccc	accatttgta	gcgccacatc	24840
ttctctttct	tcctcgctgt	ccacgattac	ctctgggtgat	ggcgggcgct	cgggcttggg	24900
agaagggcgc	ttctttttct	tcttgggcgc	aatggccaaa	tccgcgcgcg	aggtcgatgg	24960
ccgcgggctg	ggtgtgcgcg	gcaccagcgc	gtcttgtgat	gagtcctcct	cgctctcgga	25020
ctcgatacgc	cgccctcatcc	gcttttttgg	gggcgcgccg	ggaggcggcg	gcgacgggga	25080
cggggacgac	acgtcctcca	tgggtggggg	acgtcgcgc	gcaccgcgct	cgcgctcggg	25140
ggtggtttcg	cgctgctcct	cttcccgact	ggccatttcc	ttctcctata	ggcagaaaaa	25200
gatcatggag	tcagtcgaga	agaaggacag	cctaaccgcc	ccctctgagt	tcgccaccac	25260
cgctccacc	gatgccgcca	acgcgcctac	caocttcccc	gtcgaggcac	ccccgcttga	25320
ggaggaggaa	gtgattatcg	agcaggaccc	agggttttga	agcgaagacg	acgaggaccg	25380
ctcagtagca	acagaggata	aaaagcaaga	ccaggacaac	gcagaggcaa	acgaggaaac	25440
agtccggcgc	ggggacgaaa	ggcatggcga	ctacctagat	gtgggagacg	acgtgctggt	25500
gaagcatctg	cagcgccagt	gcgcgcttat	ctgcgacgcg	ttgcaagagc	gcagcgatgt	25560
gccccctgcc	atagcggatg	tcagccttgc	ctacgaacgc	cacctattct	caccgcgcgt	25620
accccccaaa	cgccaagaaa	acggcacatg	cgagcccaac	ccgcgcctca	acttctaccc	25680
cgtatttgcc	gtgccagagg	tgcttgccac	ctatcacatc	tttttccaaa	actgcaagat	25740
acccctatcc	tgccgtgcca	accgcagccg	agcggacaag	cagctggcct	tgccgcaggg	25800
cgctgtcata	cctcgctcaa	cctcgctcaa	cgaagtgcga	aaaatctttg	agggctttgg	25860
acgcgacgag	aagcgcgcg	caaacgctct	gcaacaggaa	aacagcgaaa	atgaaagtca	25920
ctctggagtg	ttggtggaac	togagggtga	caacgcgcgc	ctagccgtac	taaaacgcag	25980
catcgaggtc	acccactttg	cctacccggc	acttaacctt	cccccaagg	tcagtagcac	26040
agtcatgagt	gagctgatcg	tgcgcgctgc	gcagcccctg	gagagggatg	caaatttgca	26100
agaacaaaca	gaggagggcc	taccgcagtg	tggcgacgag	cagctagcgc	gctggcttca	26160
aacgcgcgag	cctgcccact	tggaggagcg	acgcaaacta	atgatggccg	cagtgcctgt	26220
taccgtggag	cttgagtgca	tgcagcgggt	ctttgctgac	ccggagatgc	agcgcaagct	26280
agaggaaaca	ttgcaactaca	cctttcgaca	gggtactgta	cgccaggcct	gcaagatctc	26340
caacgtggag	ctctgcaacc	tggtctccta	ccttggaatt	ttgcacgaaa	accgccttgg	26400
gcaaaacgtg	cctcattcca	cgctcaagg	cgaggcgcgc	cgcgactacg	tcgcgcactg	26460
cgtttactta	tttctatgct	acacctggca	gacggccatg	ggcgtttggc	agcagtgtct	26520
ggaggagtgc	aacctcaagg	agctgcagaa	actgctaagg	caaaacttga	aggacctatg	26580
gacggccttc	aacgagcgct	ccgtggccgc	gcacctggcg	gacatcattt	tccccgaacg	26640
cctgcttaaa	agggctcggc	agacttcacc	agacttcacc	agtcaaagca	tggtgcagaa	26700
ctttaggaac	tttatcctag	agcgctcagg	aatcttggcc	gccacctgct	gtgcacttcc	26760
tagcgacttt	gtgcccatta	agtaccgcga	atgcctccg	ccgctttggg	gccactgcta	26820
ccttctgcag	ctagccaact	accttgccct	ccactctgac	ataatggaag	acgtgagcgg	26880
tgacggtcta	ctggagtgtc	actgtcgctg	caacctatgc	accccgacc	gctccctggg	26940
ttgcaattcg	cagctgctta	acgaaaagtc	aattatcggt	acctttgagc	tgcagggtcc	27000
ctgcctgac	gaaaagtcgc	cggctccggg	gttgaaactc	actccggggc	tgtggagctc	27060

-119-

ggcttacctt	cgcaaatttg	tacctgagga	ctaccacgcc	cacgagatta	ggttctacga	27120
agaccaatcc	cgcccgccaa	atgcgaggct	taccgcctgc	gtcattaccc	agggccacat	27180
tcttgccaa	ttgcaagcca	tcaacaaagc	ccgccaagag	tttctgctac	gaaagggacg	27240
gggggtttac	ttggaccccc	agtccggcga	ggagctcaac	ccaatcccc	cgccgcccga	27300
gccctatcag	cagcagccgc	gggcccttgc	ttcccaggat	ggcaccctaa	aagaagctgc	27360
agctgccgcc	gccaccacag	gacgaggagg	aatactggga	cagtcaggca	gaggaggttt	27420
tggacgagga	ggaggaggac	atgatggaag	actgggagag	cctagacgag	gaagcttccg	27480
aggtcgaaga	ggtgtcagac	gaaacaccgt	caccctcggt	cgcatctccc	tcgcccggcg	27540
cccagaaatc	ggcaaccggt	tccagcatgg	ctacaacctc	cgctcctcag	gcgcccggcg	27600
cactgcccg	tcgcccagcc	aaccgtagat	gggacaccac	tggaaaccag	gccggtaagt	27660
ccaagcagcc	gccgcctgta	gcccagagag	aacaacagcg	ccaaggctac	cgctcatggc	27720
gcggggcaca	gaacgccata	gttgcttgc	tgcaagactg	tgggggcaac	atctccttcg	27780
ccgcgcgctt	tcttctctac	catcacggcg	tggccttccc	ccgtaacatc	ctgcattact	27840
accgtcatct	ctacagccca	tactgcaccg	gcggcagcgg	cagcggcagc	aacagcagcg	27900
gccacacaga	agcaaaggcg	accggatagc	aagactctga	caaagcccaa	gaaatccaca	27960
gcggcgggcag	cagcaggagg	aggagcgctg	cgtctggcgc	ccaacgaacc	cgtatcgacc	28020
cgcgagctta	gaaacaggat	ttttccact	ctgtatgcta	tatttcaaca	gagcaggggc	28080
caagaacaag	agctgaaaat	aaaaaacagg	tctctcgcat	ccctaccccg	cagctgcctg	28140
tatcacaaaa	gcgaagatca	gcttcggcgc	acgctggaag	acgcgagggc	tctcttcagt	28200
aaatactgcg	cgctgactct	taaggactag	tttcgcgccc	tttctcaaat	ttaagcgcg	28260
aaactacgtc	atctccagcg	gccacacccg	gcgccagcac	ctgtcgtcag	cgccattatg	28320
agcaaggaaa	ttcccacgcc	ctacatgtgg	agttaccagc	cacaaatggg	acttgcgggt	28380
ggagctgccc	aagactactc	aaccggaata	aactacatga	gcgcggggacc	ccacatgata	28440
tcccgggtca	acggaatccg	cgcccaaccg	aaccgaattc	tcttggaaca	ggcggtatt	28500
accaccacac	ctcgtataaa	ccttaatccc	cgtagttggc	ccgctgccct	ggtgtaccag	28560
gaaagtcccc	ctcccaccac	tgtggtaact	cccagagacg	cccaggccga	agttcagatg	28620
actaactcag	gggcgcagct	tgcgggcggc	tttcgtcaca	gggtgcggtc	gcccggggcag	28680
ggtataactc	acctgacaat	cagagggcga	ggtattcagc	tcaacgacga	gtcgggtgagc	28740
tctctgcttg	gtctccgtcc	ggacgggaca	tttcagatcg	gcggcgccgg	ccgtccttca	28800
ttcacgcctc	gtcaggcaat	cctaactctg	cagacctcgt	cctctgagcc	gcgctctgga	28860
ggcattggaa	ctctgcaatt	tattgaggag	tttgtgccat	cggtctactt	taaccocctc	28920
tcgggaacctc	ccggccacta	tccggatcaa	tttattccta	actttgacgc	ggtaaaggac	28980
tcggcgggacg	gctacgactg	aatgttaagt	ggagaggcag	agcaactgcg	cctgaaacac	29040
ctgggtccact	gtcgccgcca	caagtgcctt	gcccgcgact	ccggtgagtt	ttgtactttt	29100
gaattgcccc	aggatcatat	cgagggcccc	gcgcacggcg	tcgggcttac	cgcccaggga	29160
gagcttgccc	gtagcctgat	tcgggagttt	accagcgcc	ccctgctagt	tgagcgggac	29220
aggggacctc	gtgtttctac	tgtgatttgc	aactgtccta	accttggtat	acatcaagat	29280
ttaatataat	gccacatcct	cttacacttt	ttcatacatt	gcccagaagt	aaagaatcgt	29340
ttgtgttatg	tttcaacgtg	tttatttttc	aattgcagaa	aatttcaagt	catttttcat	29400
tcagtagtat	agccccacca	ccacatagct	tatacagatc	accgtacctt	aatcaaatc	29460
acagaacctc	agtattcaac	ctgccacctc	cctcccaaca	cacagagtac	acagtccttt	29520
ctccccggct	ggccttaaaa	agcatcatat	catgggtaac	agacatatct	ttaggtgtta	29580
tattccacac	ggtttctgtg	cgagccaaac	gctcatcagt	gatattaata	aactccccgg	29640
gcagctcact	taagttcatg	tcgctgtcca	gctgctgagc	cacaggctgc	tgtccaactt	29700
gcgggttgctt	aacgggcggc	gaaggagaag	tccacgccta	catgggggta	gagtcataat	29760
cgtgcatcag	gatagggcgg	tggtgctgca	gcagcgcgcg	aataaaactgc	tgccgcccgc	29820
gctccgtcct	gcaggaatac	aacatggcag	tggtctcctc	agcgatgatt	cgcacccgcc	29880
gcagcataag	gcgccttgct	ctccgggcac	agcagcgcac	cctgatctca	cttaaatcag	29940
cacagtaact	gcagcacagc	accacaatat	tgttcaaaat	cccacagtgc	aaggcgctgt	30000
atccaaagct	catggcgggg	accacagaag	ccacgtggcc	atcataccac	aagcgcatgt	30060
agattaaagt	gcgacccctc	ataaacacgc	tggacataaa	cattacctct	tttggcatgt	30120
tgtaattcac	cacctcccg	taccatataa	acctctgatt	aaacatggcg	ccatccacca	30180
ccatcctaaa	ccagctggcc	aaaacctgcc	cgccggctat	acactgcagg	gaaccggggac	30240
tggaaacaat	acagtggaga	gcccaggact	cgtaaccatg	gatcatcatg	ctcgtcatga	30300
tatcaatgtt	ggcacacac	aggcacactt	gcatacactt	cctcaggatt	acaagctcct	30360
cccgcgttag	aacctatcc	cagggaacaa	cccatctctg	aatcagcgta	aatcccacac	30420
tgcagggaag	acctcgacg	taactcacgt	tgtgcattgt	caaagtgtta	cattcgggca	30480
gcagcggatg	atcctccagt	atggtagcgc	gggtttctgt	ctcaaaagga	ggtagacgat	30540
ccctactgta	cggagtgcgc	cgagacaacc	gagatcgtgt	tggtcgtagt	gtcatgccaa	30600
atggaacgcc	ggacgtagtc	atatttctcg	aagcaaaacc	aggtgcgggc	gtgacaaaca	30660
gatctgcgtc	tccggtctcg	ccgcttagat	cgctctgtgt	agtagttgta	gtatatccac	30720

-120-

```

tctctcaaag catccaggcg ccccttggtc tcgggttcta tgtaaactcc ttcattgcgc 30780
gctgccctga taacatccac caccgcagaa taagccacac ccagccaacc tacacattcg 30840
ttctgcgagt cacacacggg aggagcggga agagctggaa gaaccatggt ttttttttta 30900
ttccaaaaga ttatccaaaa cctcaaaatg aagatctatt aagtgaacgc gctccccctc 30960
ggtggcgtgg tcaaactcta cagccaaaga acagataatg gcatttgtaa gatgttgac 31020
aatggcttcc aaaaggcaaa cggccctcac gtccaagtgg acgtaaaggc taaacccttc 31080
agggtgaatc tcctctataa acattccagc accttcaacc atgccccaaat aattctcatc 31140
tcgccacctt ctcaatatat ctctaagcaa atcccgaata cgaatcatga ttgcaaaaat 31200
aatctgctcc agagcgccct ccaccttcag cctcaagcag cgaatcatga ttgcaaaaat 31260
tcaggttcct cacagacctg tataagattc aaaagcggaa cattaacaaa aataccgcga 31320
tcccgtaggt cccttcgcag ggccagctga acataatcgt gcaggtctgc acggaccagc 31380
gcggccactt ccccgccagg aaccttgaca aaagaaccca cactgattat gacacgcata 31440
ctcggagcta tgctaaccag cgtagccccg atgtaagctt tgttgcatgg gcggcgatat 31500
aaaatgcaag gtgctgctca aaaaatcagg caaagcctcg cgcaaaaaag aaagcacatc 31560
gtagtcatgc tcatgcagat aaaggcaggt aagctccgga accaccacag aaaaagacac 31620
catttttctc tcaaacatgt ctgctgttaca acaggaaaaa caacccttat aagcataaga 31740
aacattttaa cattagaagc gtgaccgtaa aaaaactggt caccgtgatt aaaaagcacc 31800
cggactacgg cctcggctcat gtccggagtc ataatgtaag actcggtaaa cacatcaggt 31860
accgacagct gtacgtgcta aaaagcgacc gaaatagccc gggggaatac ataccgcgag 31920
tgattcatcg gctagagac aacattacag ccccataggt aggtataaca aaattaatag gagagaaaaa 31980
cacataaaca cctgaaaaac cctcctgcct aggcataaac gcaccctccc gctccagaac 32040
aacatacagc gcttcacagc ggcagcctaa cagtacgctt taccagtaaa aaagaaaacc 32100
tattaaaaaa acaccactcg acacggcacc agctcaatca gtcacagtgt aaaaaagggc 32160
caagtgcaga gcgagtatat ataggactaa aaaatgacgt aacgggttaa gtccacaaaa 32220
aacacccaga aaaccgcacg cgaacctacg cccagaaacg aaagccaaaa aaccacaaac 32280
ttcctcaaat cgtcacttcc gttttccac gttacgtaac ttcccathtt aagaaaaacta 32340
caattcccaa cacatacaag ttactccgcc ctaaaaccta cgtcacccgc cccgttccca 32400
cgccccgcgc cacgtcacia actccacccc ctctattatc tattggcttc aatccaaaat 32460
aaggtatatt attgatgatg 32480

```

<210> 107

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker Sequence

<400> 107

```

Pro Ser Ala Ser Ala Ser Ala Ser Ala Pro Gly Ser
1           5           10

```

<210> 108

<211> 8383

<212> DNA

<213> Artificial Sequence

<220>

<223> Plasmid pDV60

<400> 108

```

gacggatcgg gagatctccc gatcccctat ggtcgactct cagtacaatc tgcctctgatg 60
ccgcatagtt aagccagtat ctgtccctg cttgtgtgtt ggaggtcgct gaggtagtgcg 120
cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180
ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt 240
gattattgac tagttattaa tagtaatcaa ttacgggggtc attagttcat agcccatata 300
tggagttccg cgttacataa cttacggtaa atggcccgcg tggctgaccg cccaacgacc 360
ccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
attgacgtca atgggtggac tatttacggg aaactgccca cttggcagta catcaagtgt 480

```

-121-

atcatatgcc	aagtacgccc	cctattgacg	tcaatgacgg	taaatggccc	gcctggcatt	540
atgccagta	catgacctta	tgggactttc	ctacttggca	gtacatctac	gtattagtca	600
tcgctattac	catggtgatg	cgggttttggc	agtacatcaa	tgggcgtgga	tagcggtttg	660
actcacgggg	atctccaagt	ctccacccca	ttgacgtcaa	tgggagtttg	ttttggcacc	720
aaaatcaacg	ggactttcca	aaatgtcgta	acaactccgc	cccattgacg	caaatgggcg	780
gtaggcgtgt	acgggtgggag	gtctatataa	gcagagctct	ctggctaact	agagaaccca	840
ctgcttactg	gcttatcgaa	attaatacga	ctcactatag	ggagacccaa	gcttgggtacc	900
gagctcggat	ccactctctt	ccgcacgcgt	gtctgcgagg	gccagctgtt	ggggtagta	960
ctccctctga	aaagcgggca	tgaacttctgc	gctaagattg	tcagtttcca	aaaacgagga	1020
ggatttgata	ttcacctggc	ccgcggtgat	gcctttgagg	gtggccgcat	ccatctgggtc	1080
agaaaagaca	atctttttgt	tgtcaagcct	ggtggcaaac	gacccgtaga	gggctgtgga	1140
cagcaacttg	gcgatggagc	gcagggtttg	gtttttgtcg	cgatcggcgc	gctccttggc	1200
cgcgatgttt	agctgcacgt	attcgcgcgc	aacgcaccgc	cattcgggaa	agacgggtgg	1260
gcgctcgtcg	ggcaccagggt	gcacgcgcga	accgcggttg	tgcagggtga	caagggtcaac	1320
gctgggtggc	acctctccgc	gtaggcgctc	gttggtccag	cagaggcggc	cgcccttgcg	1380
cgagcagaat	ggcggtaggg	ggtctagctg	cgctcgtcc	ggggggctcg	cgtccacgggt	1440
aaagaccccc	ggcagcaggg	gcgcgtcgaa	gtagctctatc	ttgcatcctt	gcaagctctag	1500
cgctgctgc	catgcgcggg	cggcaagcgc	gcgctcgtat	gggttgagtg	ggggacccca	1560
tggcatgggg	tgggtgagcg	cggaggcgta	catgccgcaa	atgtcgtaaa	cgtagagggg	1620
ctctctgagt	attccaagat	atgtagggta	gcactctcca	ccgcggatgc	tggcgcgcac	1680
gtaatcgtat	agttcgtgcg	agggagcgag	gaggtcggga	ccgaggttgc	tacgggcggg	1740
gtctctgct	cgggaagacta	tctgcctgaa	gatggcatgt	gagttggatg	atatggttgg	1800
acgctggaag	acgttgaagc	tggcgtctgt	gagacctacc	gcgtcacgca	cgaaggaggc	1860
gtaggagtgc	cgcagcttgt	tgaccagctc	ggcggtgacc	tgcacgtcta	gggcgcagta	1920
gtccagggtt	tccttgatga	tgtcatactt	atcctgtccc	ttttttttcc	acagctcgcg	1980
gttagggaca	aactcttcgc	ggtcctttcca	gtactccttg	atcggaacc	cgtcggcctc	2040
cgaacgagat	ccgtactccg	ccgcgcgagg	tctagttacc	gtccgcacgc	accggatcgg	2100
aaaacctctc	gagaaaggcg	tctaaccagt	cacagtcgca	agatccaaga	tgaagcgcgc	2160
aagaccgtct	gaagatacct	tcaaccccg	gtatccatat	gacacggaaa	ccggtcctcc	2220
aactgtgcct	tttcttactc	ctccctttgt	atcccccaat	gggtttcaag	agagtcccc	2280
tgggttactc	tctttgcgcc	tatccgaacc	tctagttacc	tccaatggca	tgcttcgcgt	2340
caaaatgggc	aacggcctct	ctctggacga	ggccggcaac	cttacctccc	aaaatgtaac	2400
cactgtgagc	ccacctctca	aaaaaaccaa	gtcaaacata	aacctggaaa	tatctgcacc	2460
cctcacagtt	acctcagaag	ccctaactgt	ggctgccgcc	gcacctctaa	tggtcgcggg	2520
caacacactc	accatgcaat	cacaggcccc	gctaaccgtg	cacgactcca	aaacttagat	2580
tgccacccaa	cgacccctca	cagtgtcaga	aggaaagcta	gccctgcaaa	catcaggccc	2640
cctcaccacc	accgatagca	gtacccttac	tatcactgcc	tcacccctc	taactactgc	2700
cactggtagc	ttgggcattg	acttgaaaga	gccattttat	acacaaaatg	gaaaactagg	2760
actaaagtac	ggggctcctt	tgcattgaac	agacgacctc	aacactttga	ccgtagcaac	2820
tggtccagg	gtgactatta	ataatacttc	ccttgcaaac	aaagttactg	gagcctttgg	2880
ttttgattca	caaggcaata	tgcaacttaa	tgtagcagga	ggactaagga	ttgattctca	2940
aaacagacgc	cttatacttg	atgttagtta	tccgtttgat	gctcaaaacc	aactaaatct	3000
aagactagga	cagggccctc	tttttataaa	ctcagcccac	aacttgagta	ttaactacaa	3060
caaaggcctt	tacttgttta	cagcttcaaa	caattccaaa	aagcttgagg	ttaacctaa	3120
cactgccaag	gggttgatgt	ttgacgctac	agccatagcc	attaatgcag	gagatggggt	3180
tgaatttggt	tcacctaatg	caccaaacc	aaatcccctc	aaaacaaaaa	ttggccatgg	3240
cctagaattt	gattcaaaca	aggctatgg	tctaaacta	ggaactggcc	ttagttttga	3300
cagcacagg	gccattacag	taggaaacaa	aaataatgat	aagctaactt	tgtggaccac	3360
accagctcca	tctcctaact	gtagactaaa	tgcagagaaa	gatgctaacc	tcacttttgt	3420
cttaacaaaa	tgtggcagtc	aaataactgc	tacagttaac	gttttggtcg	ttaaaggcag	3480
tttggtcca	atatctggaa	cagttcaaac	tgtctatctt	attataagat	ttgacgaaaa	3540
tggagtgtca	ctaaacaatt	ccttcctgga	cccagaatat	tggaaactta	gaaatggaga	3600
tcttactgaa	ggcacagcct	atacaaaacg	tgtttggattt	atgcctaacc	tatcagctta	3660
tccaaaatct	cacggtaaaa	ctgccaacag	taacattgtc	agtcaagttt	acttaaacgg	3720
agacaaaaact	aaacctgtaa	cactaacac	tacactaaac	ggtacacagg	aaacaggaga	3780
cacaactcca	agtgcatact	ctatgtcatt	ttcatgggac	tggtctggcc	acaactacat	3840
taatgaaata	tttgccacat	cctcttacac	tttttcatac	attgcccagg	aataaaagaa	3900
gcggccgctc	gagcatgcat	ctagagggac	ctattctata	gtgtcaccta	aatgctagag	3960
ctcgctgac	agcctcgat	gtgccttcta	gttgccagcc	atctgttgtt	tgccctccc	4020
ccgtgccttc	cttgaccctg	gaagggtgca	ctcccactgt	cctttcctaa	taaaatgagg	4080
aaattgcatc	gcattgtctg	agtaggtgtc	attctattct	gggggggtgg	gtggggcagg	4140

-122-

acagcaaggg	ggaggattgg	gaagacaata	gcaggcatgc	tggggatgcg	gtgggctcta	4200
tggcttctga	ggcggaaga	accagctggg	gctctagggg	gtatccccac	gcgccctgta	4260
gcggcgcat	aagcgcgcg	ggtgtggtgg	ttacgcgcag	cgtgaccgct	acacttgcca	4320
gcgccctagc	gcccgcctct	ttcgctttct	tcccttcctt	tctcgccacg	ttcgccggct	4380
ttccccgtca	agctctaaat	cggggcatcc	ctttaggggt	ccgatttagt	gctttacggc	4440
acctcgaccc	caaaaaactt	gattaggggt	atggttcacg	tagtgggcca	tgcgccgat	4500
agacggtttt	tgcgcctttg	acgttggagt	ccacgttctt	taatagtga	ctcttgttcc	4560
aaactggaac	aacactcaac	cctatctcgg	tctattcctt	tgatttataa	gggattttgg	4620
ggatttcggc	ctattgggta	aaaaatgagc	tgatttaaca	aaaatttaac	gcgaattaat	4680
tctgtggaat	gtgtgtcagt	taggggtgtg	aaagtcccca	ggctccccag	gcaggcagaa	4740
gtatgcaaag	catgcctctc	aattagtcag	caaccagggt	tggaaagtcc	ccaggctccc	4800
cagcaggcag	agcatgcata	ctaatgcctc	tcaattagtc	agcaaccata	gtcccgcccc	4860
taactccgcc	catcccgccc	ctaactccgc	ccagtccgcg	ccattctccg	ccccatggct	4920
gactaatttt	ttttatttat	gcagaggccg	aggccgcctc	tgcctctgag	ctattccaga	4980
agtagtgagg	aggctttttt	ggaggccctag	gcttttgcaa	aaagctcccc	ggagcttgta	5040
tatccatttt	cggatctgat	caagagacag	gatgaggatc	gtttcgcagt	attgaacaag	5100
atggattgca	cgcaggttct	ccggccgctt	gggtggagag	gctattcggc	tatgactgaa	5160
cacaacagac	aatcggtctg	tctgatgccg	cggtgttccg	gctgtcagcg	caggggcgcc	5220
cggttctttt	tgtcaagacc	gacctgtccg	gtgccctgaa	tgaactgcag	gacgaggcag	5280
cgcggctatc	gtggctggcc	acgacgggcg	ttccttgccg	agctgtgctc	gacgttgta	5340
ctgaagcggg	aaggagctgg	ctgctattgg	gcgaagtgcc	ggggcaggat	ctcctgtcat	5400
ctcaccttgc	tcttcgagag	aaagtatcca	tcatggctga	tgcaatgcgg	cggctgcata	5460
cgcttgatcc	ggctacctgc	ccattcgacc	accaagcgaa	acatcgcatc	gagcgagcac	5520
gtactcggat	ggaagccggt	cttgtcgatc	aggatgatct	ggacgaagag	catcaggggc	5580
tcgcgccagc	cgaactgttc	gccaggctca	aggcgcgcat	gcccgaaggc	gaggatctcg	5640
tcgtgaccca	tggcgatgcc	tgcttgccga	atatcatggt	ggaaaatggc	cgcttttctg	5700
gattcatcga	ctgtggcccg	ctgggtgtgg	cggaccgcta	tcaggacata	gcgttggtta	5760
cccgtgatat	tgtcgaagag	cttggcggcg	aatgggctga	ccgcttctct	gtgctttacg	5820
gtatcgccgc	tcccgattcg	cagcgcatcg	ccttctatcg	ccttcttgac	gagttcttct	5880
gagcgggact	ctgggggttcg	aaatgaccga	ccaagcgacg	cccaacctgc	catcacgaga	5940
tttcgatttc	acgcgcgctt	tctatgaaag	gttgggcttc	ggaatcgttt	tccgggacgc	6000
cggctggatg	atcctccagc	gcggggatct	catgctggag	ttcttcgccc	accccaactt	6060
gtttattgca	gcttataatg	gttacaaata	aagcaatagc	atcacaaatt	tcacaaataa	6120
agcatttttt	tcaactgcatt	ctagttgtgg	tttgtccaaa	ctcatcaatg	tatcttatca	6180
tgtctgtata	cogtcgacct	ctagctagag	cttggcgtaa	tcattggtcat	agctgtttcc	6240
tgtgtgaaat	tgttatccgc	tcacaattcc	acacaacata	cgagccggaa	gcataaagtg	6300
taaagcctgg	ggtgcctaata	gagtgcgcta	actcacatta	attgcggtgc	gctcactgcc	6360
cgctttccag	tccgggaaacc	tgtcgtgccg	gctgcattaa	tgaatcggcc	aacgcgcggg	6420
gagaggcggg	ttgcgtattg	ggcgcctctc	cgcttccctc	ctcactgact	cgctgcgctc	6480
ggctcgttcg	ctgcggcgag	cggtatcagc	tactcaaaag	gcggtaatac	ggttatccac	6540
agaatcaggg	gataagcgag	gaaagaacat	gtgagcaaaa	ggccagcaaa	aggccaggaa	6600
ccgtaaaaaag	gcgcggttgc	tggcgttttt	ccataggctc	cgccccctcg	acgagcatca	6660
caaaaaatcga	cgctcaagtc	agaggtggcg	aaaccgcaga	ggactataaa	gataccaggc	6720
gtttccccct	ggaagctccc	tctgtgcgctc	tctgttcccg	accctgcccg	ttaccggata	6780
cctgtccgcc	ttctccctt	cgggaaagcg	ggcgctttct	caatgctcac	gctgtaggta	6840
tctcagttcg	gtgtagggtc	ttcgctccaa	gctgggctgt	gtgcacgaac	cccccgttca	6900
gcccgcaccg	tgcgccttat	ccgtaacta	tctgtctgag	tccaaccggg	taagacacga	6960
cttatcgcca	ctggcagcag	ccactggtaa	caggattagc	agagcgagg	atgtaggcgg	7020
tgctacagag	ttcttgaagt	ggtggcctaa	ctacggctac	actagaagga	cagtatttgg	7080
tatctgcgct	tctgtgaagc	cagttacctt	cggaaaaaga	gttggttagct	cttgatccgg	7140
caaacaaacc	accgctggta	gcggtgggtt	ttttgtttgc	aagcagcaga	ttacgcgcag	7200
aaaaaaagga	tctcaagaag	atcctttgat	cttttctacg	gggtctgacg	ctcagtggaa	7260
cgaaaactca	cgtaaaggga	ttttgggtcat	gagattatca	aaaaggatct	tcacctagat	7320
ccttttaaat	taaaaatgaa	gttttaaatc	aatctaaagt	atatatgagt	aaacttggtc	7380
tgacagttac	caatgcttaa	tcagtggagg	acatctctca	gcgatctgtc	tatttcggtc	7440
atccatagtt	gcctgactcc	ccgtcgtgta	gataactacg	atacgggagg	gcttaccatc	7500
tggccccagt	gctgcaatga	taccgcgaga	cccacgctca	ccggctccag	atztatcagc	7560
aataaaccag	ccagccggaa	gggcccagcg	cagaagtggg	cctgcaactt	tatccgcctc	7620
catccagctc	attaattggt	gcccgggaag	tagagtaagt	agttcgccag	ttaatagttt	7680
gcgcaacggt	gttgccattg	ctacaggcat	cgtggtgtca	cgctcgtcgt	ttggtatggc	7740
ttcattcagc	tccggttccc	aacgatcaag	gcgagttaca	tgatccccc	tgttgtgcaa	7800

-123-

```

aaaagcgggt agctccttcg gtcctccgat cgttggtcaga agtaagttgg ccgcagtgtt 7860
atcactcatg gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg 7920
cttttctgtg actgggtgag actcaaccaa gtcattctga gaatagtgtg tgcggcgacc 7980
gagttgctct tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa 8040
agtgtctcatc attggaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt 8100
gagatccagt tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt 8160
caccagcgtt tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa agggaataag 8220
ggcgacacgg aaatgttgaa tactcatact cttccttttt caatattatt gaagcattta 8280
tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat 8340
aggggttccg cgcacatttc cccgaaaagt gccacctgac gtc 8383

```

<210> 109

<211> 7960

<212> DNA

<213> Artificial Sequence

<220>

<223> Plasmid pDV67

<400> 109

```

gacggatcgg gagatctccc gatccccctat ggtcgactct cagtacaatc tgctctgatg 60
ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg 120
cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180
ttaggggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatatc cgttgacatt 240
gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata 300
tggagttccg cgttacataa cttacggtaa atggcccgc tggctgaccg cccaacgacc 360
ccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
attgacgtca atgggtggac tatttacggt aaactgcca cttggcagta catcaagtgt 480
atcatatgcc aagtacgcc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540
atgccagta catgacctta tgggactttc ctacttgga gtacatctac gtattagtca 600
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 660
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720
aaaatcaacg ggactttcca aaatgtcgta acaactccgc ccattgacg caaatgggcg 780
gtaggcgtgt acgggtgggag gtctatataa gcagagctct ctggctaact agagaaccca 840
ctgcttactg gcttatcgaa attaatacga ctactatag ggagacccaa gctggctagc 900
gtttaaactt aagcttggtg cagagctcgg atccactctc ttccgcatcg ctgtctgcga 960
gggccagctg ttgggggtgag tactccctct gaaaagcggg catgacttct gcgctaagat 1020
tgtcagtttc caaaaacgag gaggatttga tattcacctg gcccgcggtg atgcctttga 1080
gggtggccgc atccatctgg tcagaaaaga caatcttttt gttgtcaagc ttggtggcaa 1140
acgacccgta gagggcggtg gacagcaact tggcgatgga gcgcagggtt tggtttttgt 1200
cgcgctcggc gcgctccttg cccgcgatgt tttagctcac gtattcgcgc gcaacgcacc 1260
gccattcggg aaagacgggt gtgcgctcgt cgggcaccag gtgcacgcgc caaccgcggt 1320
tgtgcagggt gacaagggtca acgctggtgg ctacctctcc gcgtaggcgc tcgttgggtcc 1380
agcagaggcg gccgcccttg cgcgagcaga atggcggtag ggggtctagc tgcgtctcgt 1440
ccgggggggtc tgcgtccacg gtaaaagacc cgggcagcag gcgcgcgtcg aagtagtcta 1500
tcttgcatcc ttgcaagtct agcgcctgct gccatgcgcg ggccggcaagc gcgcgctcgt 1560
atggggttag tgggggaccc catggcatgg ggtgggtgag cgcggaggcg tacatgccgc 1620
aaatgtcgta aacgtagagg ggctctctga gtattccaag atatgtaggg tagcatcttc 1680
caccgcgatg gctggcgcg cgtaatcgt atagttcgtg cgagggagcg aggaggtcgg 1740
gaccgaggtt gctacgggcg ggctgctctg ctcggaagac tatctgcctg aagatggcat 1800
gtgagttgga tgatatggtt ggacgctgga agacgttgaa gctggcgtct gtgagacct 1860
ccgcgtcacg cacgaaggag gcgtaggagt cgcgcagctt gttgaccagc tcggcggtga 1920
cctgcacgtc tagggcgag tagtccaggg tttccttgat gatgtcatac ttatcctgtc 1980
cctttttttt ccacagctcg cggttgagga caaactcttc gcggtcttcc cagtactctt 2040
ggatcggaaa cccgtcggcc tccgaacgag atccgtactc cgccgcccag ggacctgagc 2100
gagtcgcgat cgaccggatc ggaaaacctc tcgagaaagg cgtctaacca gtcacagtcg 2160
caagatccaa gatgaagcgc gcaagaccgt ctgaagatac cttcaacccc gtgtatccat 2220
atgacacgga aaccggctct ccaactgtgc cttttcttac tccctccctt gtatccccc 2280
atgggtttca agagagtcct cctggggtag tctctttgag cctatccgaa cctctagtta 2340
cctccaatgg catgcttgcg ctcaaaatgg gcaacggcct ctctctggac gaggccggca 2400
accttacctc ccaaaatgta accactgtga gccacctct caaaaaacc aagtcaaaca 2460

```


-124-

taaacctgga	aatatctgca	ccccctcacag	ttacctcaga	agccctaact	gtggctgccc	2520
cgcacacctc	aatggctcg	ggcaacacac	tcaccatgca	atcacaggcc	ccgctaaccg	2580
tgacgactc	caaacttagc	attgccaccc	aaggacccct	cacagtgtca	gaaggaaagc	2640
tagccctgca	aacatcaggc	ccccctacca	ccaccgatag	cagtaccctt	actatcactg	2700
cctcaccccc	tctaactact	gccactggta	gcttgggcat	tgacttgaaa	gagcccat	2760
atacacaaaa	tggaaaacta	ggactaaagt	acggggctcc	tttgcatgta	acagacgacc	2820
taaacacttt	gaccgtagca	actgggtccag	gtgtgactat	taataatact	tccttgcaaa	2880
ctaaagttac	tggagccttg	ggtttttgatt	cacaaggcaa	tatgcaactt	aatgtagcag	2940
gaggactaag	gattgattct	caaaacagac	gccttatact	tgatgttagt	tatccgtttg	3000
atgctcaaaa	ccaactaaat	ctaagactag	gacagggccc	tctttttata	aactcagccc	3060
acaacttgga	tattaactac	aacaaaggcc	tttacttggt	tacagcttca	aacaattcca	3120
aaaagcttga	ggttaacctg	agcactgcca	aggggttgat	gtttgacgct	acagccatag	3180
ccattaatgc	aggagatggg	cttgaatttg	gttcaccta	tgacacaaac	acaaatcccc	3240
tcaaaacaaa	aattggccat	ggcctagaat	ttgattcaaa	caaggctatg	gttcctaaac	3300
taggaactgg	ccttagtttt	gacagcacag	gtgccattac	agtaggaaac	aaaaataatg	3360
ataagctaac	tttgtggacc	acaccagctc	catctcctaa	ctgtagacta	aatgcagaga	3420
aagatgctaa	actcaccttg	gtcttaacaa	aatgtggcag	tcaaataact	gctacagttt	3480
cagttttggc	tgttaaaggc	agtttggctc	caatatctgg	aacagttcaa	agtgtctcat	3540
ttattataag	atttgacgaa	aatggagtgc	tactaaacaa	ttccttcctg	gacccagaat	3600
attggaactt	tagaaatgga	gatcttactg	aaggcacagc	ctatacaaac	gctgttggat	3660
ttatgcctaa	cctatcagct	tatccaaaat	ctcacggtaa	aactgccaaa	agtaacattg	3720
tcagtcaagt	ttacttaaac	ggagacaaaa	ctaaacctgt	aacactaacc	attacactaa	3780
acggtacaca	ggaaacagga	gacacaactc	caagtgcata	ctctatgtca	ttttcatggg	3840
actggctctg	ccacaactac	attaatgaaa	tatttgccac	atcctcttac	actttttcat	3900
acattgcccc	agaataaaaag	aagcggccgc	tcgagtctag	agggcccgtt	taaaccocgt	3960
gatcagctc	gactgtgcct	tctagtggc	agccatctgt	tgtttgcccc	ttccccgtgc	4020
cttccttgac	cctggaaggt	gccactccca	ctgtcctttc	ctaataaaaat	gaggaaattg	4080
catcgcatg	tctgagtagg	tgtcattcta	ttctgggggg	tggggtgggg	caggacagca	4140
agggggagga	ttgggaagac	aatagcaggc	atgctgggga	tgcggtgggc	tctatggott	4200
ctgaggcgga	aagaaccagc	tggggctcta	gggggtatcc	ccacgcgccc	tgtagcgggc	4260
cattaagcgc	ggcgggtgtg	gtgggttacgc	gcagcgtgac	cgctacactt	gccagcgccc	4320
tagcgcggc	tcctttcgct	ttcttccctt	ccttctctgc	cacgttcgcc	ggctttcccc	4380
gtcaagctct	aaatcggggc	atcccttttag	ggttccgatt	tagtgcttta	cggcacctcg	4440
acccccaaaa	acttgattag	ggtgatgggt	cacgtagtgg	gccatcgccc	tgatagacgg	4500
tttttgcgcc	tttgacgttg	gagtccacgt	tctttaatag	tggaactctg	ttccaaactg	4560
gaacaacact	caaccctatc	tcgggtctatt	cttttgattt	ataagggatt	ttggggattt	4620
cggcctattg	gttaaaaaat	gagctgattt	aacaaaaatt	taacgcgaat	taattctgtg	4680
gaatgtgtgt	cagttagggg	gtggaaagtc	cccaggctcc	ccaggcaggc	agaagtatgc	4740
aaagcatgca	tctcaattag	tcagcaacca	ggtgtggaaa	gtccccaggc	ttcccagcag	4800
gcagaagatg	gcaaagcatg	catctcaatt	agtcagcaac	catagtcccg	cccctaactc	4860
cgcccatccc	gccccactac	cgccccagtt	ccgcccatct	tccgccccat	ggctactata	4920
ttttttttat	ttatgcagag	gcgcaggccg	cctctgcctc	tgagctatct	cagaagtagt	4980
gaggaggctt	ttttggaggc	ctaggctttt	gcaaaaagct	cccgggagct	tgtatatcca	5040
ttttcggatc	tgatcagcac	gtgttgacaa	ttaatcatcg	gcatagtata	tcggcatagt	5100
ataatacgac	aaggtaggga	actaaaccat	ggccaagttg	accagtgcgc	ttccggtgct	5160
caccgcgcgc	gacgtcgccg	gagcgggtcg	gttctggacc	gaccggctcg	ggttctcccg	5220
ggacttcgtg	gaggacgact	tcgcgggtgt	ggtccgggac	gacgtgacc	tgttcatcag	5280
cgcgggtccag	gaccaggtgg	tgccggacaa	caccctggcc	tggtgtgggg	tgccggccct	5340
ggacgagctg	tacgcgaggt	ggtcggaggt	cgtgtccacg	aacttccggg	acgcctcccg	5400
gccggccatg	accgacatcg	gcgagcagcc	gtggggcgcg	gagttcgccc	tgccgcagcc	5460
ggccggcaac	tgctgacact	tcgtggccga	ggagcaggac	tgacacgtgc	tacgagattt	5520
cgattccacc	gccgccttct	atgaaagggt	gggcttcgga	atcgttttcc	gggacgcggg	5580
ctggatgatc	ctccagcgcg	gggatctcat	gctggagttc	ttcgccacc	ccaacttggt	5640
tattgcagct	tataattggt	acaaataaag	caatagcatc	acaaatttca	caaataaagc	5700
atttttttca	ctgcattcta	gttgtggttt	gtccaaactc	atcaatgtat	cttatcatgt	5760
ctgtataccg	tcgacctcta	gctagagctt	ggcgtaatca	tggtcatagc	tgtttctctgt	5820
gtgaaattgt	tatccgctca	caattccaca	caacatacga	gccggaagca	taaagtgtaa	5880
agcctggggg	gcctaattgag	tgagctaact	cacattaatt	gcgttgccgt	cactgcccgc	5940
tttccagtcg	ggaaacctgt	cgtgccagct	gcattaatga	atcgcccaac	gcgcggggag	6000
aggcgggttg	cgtattgggc	gctcttccgc	ttctctcgct	actgactcgc	tgccgtcggt	6060
cgttcggctg	cggcgagcgg	tatcagctca	ctcaaaggcg	gtaatacggg	tatccacaga	6120

-125-

```

tcaggggat aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg 6180
aaaaaaggcc gcgttgctgg cgtttttcca taggctccgc cccctgacg agcatcacaa 6240
aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcggt 6300
tccccctgga agctccctcg tgcgctctcc tggtccgacc ctgccgctta ccggatacct 6360
gtccgccttt ctcccttcgg gaagcgtggc gctttctcaa tgctcacgct gtaggtatct 6420
cagttcgggtg taggtcggtc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc 6480
cgaccgctgc gccttatccg gtaactatcg tcttgagtcc aacccggtaa gacacgactt 6540
atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc 6600
tacagagttc ttgaagtggg ggcctaacta cggctacact agaaggacag tatttggtat 6660
ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa 6720
acaaaccacc gctggtagcg gtgggttttt tgtttgcaag cagcagatta cgcgagaaa 6780
aaaaggatct caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaaacga 6840
aaactcacgt taagggatth tggtcatgag attatcaaaa aggatcttca cctagatcct 6900
tttaaattaa aaatgaagtt ttaaataaat cttaaagtata tatgagtaaa cttggtctga 6960
cagttaccaa tgcttaatac gtgaggcacc tatctcagcg atctgtctat ttcgttcatc 7020
catagttgcc tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg 7080
cccagtgct gcaatgatac cgcgagaccc acgctcaccg gctccagatt tatcagcaat 7140
aaaccagcca gccggaaggg ccgagcgagc aagtggctct gcaactttat ccgcctccat 7200
ccagtctatt aattgttgcc gggaagctag agtaagtagt tcgccagtta atagtttgcg 7260
caacgttggt gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc 7320
attcagctcc ggttcccaac gatcaaggcg agttacatga tcccccatgt tgtgcaaaaa 7380
agcggttagc tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc 7440
actcatgggt atggcagcac tgcataatth tcttactgtc atgccatccg taagatgctt 7500
ttctgtgact ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag 7560
ttgctcttgc ccggcgtaaa tacgggataa taccgcgcca catagcagaa ctttaaaagt 7620
gctcatcatt ggaaaacggt cttcggggcg aaaactctca aggatcttac cgctgttgag 7680
atccagttcg atgtaaccca ctcgtagacc caactgatct tcagcatctt ttactttcac 7740
cagcgtttct ggggtgagcaa aaacaggaag gcaaaaatgcc gcaaaaaagg gaataagggc 7800
gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa gcatttatca 7860
gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg 7920
ggttccgcgc acatttcccc gaaaagtgc acctgacgtc 7960

```

<210> 110

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> pGEM5TS3H forward primer

<400> 110

atgggatcca agatgaagcg cgcaagaccg

30

<210> 111

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> pGEM5TS3H reverse primer

<400> 111

cactatagcg gccgcattct cagtcattct

30

<210> 112

<211> 7989

<212> DNA

<213> Artificial Sequence

<220>

<223> Plasmid pDV69

-126-

<221> misc_feature
 <222> 4242,4245
 <223> n = A,T,C or G

<400> 112

gacggatcgg	gagatctccc	gatccccctat	ggtecgactct	cagtacaatc	tgctctgatg	60
ccgcatagtt	aagccagtat	ctgctccctg	cttgtgtgtt	ggaggtcgct	gagtagtgcg	120
cgagcaaaat	ttaagctaca	acaaggcaag	gcttgaccga	caattgcatg	aagaatctgc	180
ttaggggttag	gcgtttttgcg	ctgcttcgcg	atgtacgggc	cagatatacg	cgttgacatt	240
gattattgac	tagttattaa	tagtaatcaa	ttacgggggtc	attagttcat	agcccatata	300
tggagttccg	cgttacataa	cttacggtaa	atggcccgc	tggctgaccg	cccaacgacc	360
cccgcacatt	gacgtcaata	atgacgtatg	ttcccatagt	aacgccaata	gggactttcc	420
attgacgtca	atgggtggac	tatttacggg	aaactgccc	cttggcagta	catcaagtgt	480
atcatatgcc	aagtacgccc	cctattgacg	tcaatgacgg	taaatggccc	gcctggcatt	540
atgcccagta	catgacctta	tgggactttc	ctacttggca	gtacatctac	gtattagtca	600
tgcgtattac	catggtgatg	cggtttttggc	agtacatcaa	tgggcgtgga	tagcggtttg	660
actcacgggg	atttccaagt	ctccacccca	ttgacgtcaa	tgggagtttg	ttttggcacc	720
aaaatcaacg	ggactttcca	aaatgtcgta	acaactccgc	cccatgtacg	caaatgggcg	780
gtaggcgtgt	acgggtgggag	gtctatataa	gcagagctct	ctggctaact	agagaaccca	840
ctgcttactg	gcttatcgaa	attaatacga	ctcactatag	ggagacccaa	gctggctagc	900
gttttaaactt	aagcttggta	ccgagctcgg	atccactctc	ttccgcacgc	ctgtctgcga	960
gggcccagctg	ttgggggtgag	tactccctct	gaaaagcggg	catgacttct	gcgctaagat	1020
tgtcagtttc	caaaaacgag	gaggatttga	tattcacctg	gcccgcggtg	atgcctttga	1080
gggtggccgc	atccatctgg	tcagaaaaga	caatcttttt	gttgtcaagc	ttgggtggcaa	1140
acgacccgta	gagggcggtt	gacagcaact	tggcgatgga	gcgcagggtt	tggtttttgt	1200
cgcgatcggc	gcgctccttg	gccgcgatgt	ttagctgcac	gtattcgcgc	gcaacgcacc	1260
gccattcggg	aaagacggtg	gtgcgctcgt	cgggcaccag	gtgcacgcgc	caaccgcggt	1320
tgtgcagggg	gacaagggtca	acgctgggtg	ctacctctcc	gcgtaggcgc	tcgttgggtcc	1380
agcagaggcg	gccgcccttg	cgcgagcaga	atggcggtag	ggggtctagc	tgcgctctcgt	1440
ccgggggggtc	tgcgctccacg	gtaaagaccc	cgggcagcag	gcgcgcgctc	aagtagtcta	1500
tcttgcatcc	ttgcaagtct	agcgcctgct	gccatgcgcg	ggcggcaagc	gcgcgctcgt	1560
atgggttgag	tgggggaccc	catggcatgg	gggtgggtgag	cgcgagggcg	tacatgccgc	1620
aaatgtcgta	aacgtagagg	ggctctctga	gtattccaag	atatgtaggg	tagcatcttc	1680
caccgcggat	gctggcgcg	acgtaatcgt	atagttcgtg	cgagggagcg	aggaggtcgg	1740
gaccgaggtt	gctacggg	ggctgctctg	ctcggaagac	tatctgcctg	aagatggcat	1800
gtgagttgga	tgatattggt	ggacgctgga	agacgttgaa	gctggcgtct	gtgagacctt	1860
ccgcgtcacg	cacgaaggag	gcgtaggagt	cgcgacgctt	gttgaccagc	tcggcggtga	1920
cctgcacgtc	tagggcgag	tagtccaggg	tttccctgat	gatgtcatac	ttatcctgtc	1980
cctttttttt	ccacagctcg	cggttgagga	caaactcttc	gcggtctttc	cagtaactctt	2040
ggatcggaaa	cccgtcggcc	tcggaacgag	atcogtaact	cgccgccgag	ggacctgagc	2100
gagtcgcgat	cgaccggatc	ggaaaacctc	tcgagaaaag	cgtctaacca	gtcacagtgc	2160
caagatccaa	gatgaagcgc	gcaagaccgt	ctgaagatac	cttcaacccc	gtgtatccat	2220
atgacacgga	aaccggctct	ccaactgtgc	cttttcttac	tcctcccttt	gtatccccc	2280
atgggtttca	agagagtccc	cctgggggtac	tctcttttgcg	cctatccgaa	cctctagtta	2340
cctccaatgg	catgcttgcg	ctcaaaatgg	gcaacggcct	ctctctggac	gaggccggca	2400
accttacctc	ccaaaatgta	accactgtga	gcccacctct	caaaaaaacc	aagtcaaaca	2460
taaaacctgga	aatatctgca	cccctcacag	ttacctcaga	agccctaact	gtggctgcgc	2520
ccgcacctct	aatggctcgc	ggcaacacac	tcacctgca	atcacaggcc	ccgctaaccg	2580
tgcacgactc	caaacttagc	attgcccacc	aaggacccct	cacagtgtca	gaaggaaaagc	2640
tagccctgca	aactcaggc	cccctcacca	ccaccgatag	cagtaccctt	actactactg	2700
cctcaccccc	cttaactact	gccactggta	ctttgggcat	tgacttgaaa	gagccattt	2760
atacacaaaa	tggaaaacta	ggactaaagt	acggggctcc	tttgcatgta	acagacgacc	2820
taaaactttt	gaccgtagca	actggtccag	gtgtgactat	taataatact	tccttgcaaa	2880
ctaaagttac	tggagccttg	ggtttttgatt	cacaaggcaa	tatgcaactt	aatgtagcag	2940
gaggactaag	gattgattct	caaaaacagac	gccttatact	tgatgttagt	tatccgtttg	3000
atgctcaaaa	ccaactaaat	ctaagactag	gacagggccc	tctttttata	aactcagccc	3060
acaacttgga	tattaactac	aacaaaggcc	tttacttggt	tacagcttca	aacaattcca	3120
aaaagcttga	ggttaaccta	agcactgcca	aggggttgat	gtttgacgct	acagccatag	3180
ccattaatgc	aggagatggg	cttgaatttg	gttcacctaa	tgaccaaac	acaaatcccc	3240
tcaaaaacaaa	aattggccat	ggcctagaat	ttgattcaaa	caaggctatg	gttcctaacc	3300
taggaactgg	ccttagtttt	gacagcacag	gtgccattac	agtaggaaac	aaaaataatg	3360

-127-

ataagctaac	tttgtggacc	ggtccaaaac	cagaagccaa	ctgcataatt	gaatacggga	3420
aacaaaaccc	agatagcaaa	ctaactttaa	tccttgtaaa	aaatggagga	attgttaaatg	3480
gatatgtaac	gctaattggga	gcctcagact	acgttaacac	cttattttaaa	aacaaaaatg	3540
tctccattaa	tgtagaacta	tactttgatg	ccactgggtca	tatattacca	gactcatctt	3600
ctcttaaaac	agatctagaa	ctaaaataca	agcaaaccgc	tgacttttagt	gcaagagggtt	3660
ttatgccaaag	tactacagcg	tatccatttg	tccttcctaa	tgcggggaaca	cataatgaaa	3720
attatatTTTT	tggtcaatgc	tactacaaaag	caagcgatgg	tgccctTTTT	ccggtgggaag	3780
ttactgttat	gcttaataaaa	cgcttgccag	atagtcgcac	atcctatgtt	atgactTTTT	3840
tatggctcctt	gaatgctggg	ctagctccag	aaactactca	ggcaaccctc	ataacctccc	3900
catttacctt	ttcctatat	agagaagatg	actgattttt	aagaagcggc	cgctcgagtc	3960
tagagggccc	gtttaaaccc	gctgatcagc	ctcgactgtg	ccttctagtt	gccagccatc	4020
tggtgtttgc	ccctcccccg	tgcttccctt	gacctgggaa	ggtgccactc	ccactgtcct	4080
ttcctaataaa	aatgaggaaa	ttgcatcgca	ttgtctgagt	aggtgtcatt	ctattctggg	4140
gggtgggggtg	gggcaggaca	gcaagggggga	ggattgggaa	gacaatagca	ggcatgctgg	4200
ggatgcgggtg	ggctctatgg	cttctgaggg	ggaaagaacc	snccttagct	ggggctctag	4260
gggggtatccc	cacgcgcctt	gtagcggcgc	attaagcgcg	gcgggtgtgg	tggttacgcg	4320
cagcgtgacc	gctacacttg	ccagcgcctt	agcgcgcctt	cctttcgctt	tcttcccttc	4380
ctttctcgcc	acgttcgcgc	gctttccccc	tcaagctcta	aatcggggca	tccctttagg	4440
gttccgattt	agtgtcttac	ggcacctcga	ccccaaaaaa	cttgattagg	gtgatgggtc	4500
acgtagtggg	ccatcgccct	gatagacggg	ttttcgccct	ttgacgttgg	agtccacgtt	4560
ctttaatagt	ggactcttgt	tccaaactgg	aacaacactc	aaccctatct	cggtctattc	4620
ttttgattta	taagggtttt	tggggatttc	ggcctatttg	ttaaaaaatg	agctgattta	4680
acaaaaatTT	aacgcgaatt	aattctgtgg	aatgtgtgtc	agttagggtg	tggaaagtcc	4740
ccaggctccc	caggcaggca	gaagtatgca	aagcatgcat	ctcaattagt	cagcaaccag	4800
gtgtggaaaag	tccccagggt	ccccagcagg	cagaagtatg	caaagcatgc	atctcaatta	4860
gtcagcaacc	atagtcccgc	ccctaactcc	gcccattccc	cccctaactc	cgcccagttc	4920
cgcccatctt	cgcccccag	gctgactaat	tttttttatt	tatgcagagg	ccgagggcgc	4980
ctctgcctct	gagctattcc	agaagtagtg	aggaggcttt	tttgagggcc	taggcttttg	5040
caaaaagctc	ccgggagctt	gtatatccat	tttcggatct	gatcagcacg	tggtgacaat	5100
taatcatcgg	catagtatat	cggcatagta	taatacgaca	aggtgaggaa	ctaaaccatg	5160
gccaaagtta	ccagtgcctg	tccgggtgct	accgcgcgcg	acgtcgccgg	agcgggtcgag	5220
ttctggaccg	accggctcgg	gttctcccg	gacttcgtgg	aggacgactt	cgccggtgtg	5280
gtccggggacg	acgtgaccct	gttcatcagc	gcgggtccagg	accagggtgt	gccggacaac	5340
accctggcct	gggtgtgggt	gcgcggcctg	gacgagctgt	acgccagagt	gtcggagggtc	5400
gtgtccacga	acttccggga	cgcctccggg	ccggccatga	ccgagatcgg	cgagcagccg	5460
tggggggcggg	agttcgccct	gcgcgaaccg	gccggcaact	gcgtgcactt	cgtggccgag	5520
gagcaggact	gacacgtgct	acgagatttc	gattccaccc	cgccttcta	tgaaggtttg	5580
ggcttcggaa	tcgttttccg	ggacgcgggc	tggatgatcc	tccagcgcgg	ggatctcatg	5640
ctggagttct	tcgcccaccc	caacttgttt	attgcagctt	ataatggtta	caaataaagc	5700
aatagcatca	caaatttcac	aaataaagca	tttttttcac	tgcatcttag	ttgtggtttg	5760
tccaaactca	tcaatgtatc	ttatcatgtc	tgtataccgt	cgacctctag	ctagagcttg	5820
gcgtaatcat	ggtcatagct	gtttcctgtg	tgaattgttt	atccgctcac	aattccacac	5880
aacatacgag	ccggaagcat	aaagtgtaaa	gcctgggggtg	cctaattgagt	gagctaactc	5940
acattaattg	cgttgcgctc	actgcccgtt	ttccagtcgg	gaaacctgtc	gtgccagctg	6000
cattaatgaa	tcggccaacg	cgcggggaga	ggcggtttgc	gtattggggc	ctcttccgct	6060
tctcgtctca	ctgactcgct	gcgctcggtc	gttcggctgc	ggcgagcggg	atcagctcac	6120
tcaaaggcgg	taatacgggt	atccacagaa	tcagggggata	acgcaggaaa	gaacatgtga	6180
gcaaaaggcc	agcaaaaggc	caggaaccgt	aaaaaggccg	cgttgctggc	gtttttccat	6240
aggctccgcc	ccctgacga	gcatacaaaa	aatcgacgct	caagtcagag	gtggcgaaac	6300
ccgacaggac	tataaagata	ccaggcgttt	ccccctggaa	gctccctcgt	gcgctctcct	6360
gttccgaccc	tgcgcttac	cggatacctg	tccgcttttc	tcccttcggg	aagcttggcg	6420
ctttctcaat	gctcacgctg	taggtatctc	agttcggtgt	aggtcgttcg	ctccaagctg	6480
ggctgtgtgc	acgaaccccc	cgttcagccc	gaccgctgcg	ccttatccgg	taactatcgt	6540
cttgagtcca	acccggttaag	acacgactta	tcgcccactgg	cagcagccac	tggttaacagg	6600
attagcagag	aggcggtgtg	aggcggtgtt	acagagttct	tgaagtgggtg	gcctaactac	6660
ggctacacta	gaaggacagt	atttgggtatc	tcgctctctg	tgaagccagt	taccttcgga	6720
aaaagagtgt	gtagctcttg	atccggcaaa	caaaccaccg	ctggtagcgg	tggttttttt	6780
gtttgcaagc	agcagattac	gcgcagaaaa	aaaggatctc	aagaagatcc	tttgatcttt	6840
tctacggggg	ctgacgctca	gtggaacgaa	aactcacgtt	aagggtatttt	ggtcatgaga	6900
ttatcaaaaa	ggatcttcac	ctagatcctt	ttaaattaaa	aatgaagttt	taaataaatc	6960
taaagtatat	atgagtaaac	ttggtctgac	agttaccaat	gcttaatcag	tgaggcacct	7020

-128-

```

atctcagcga tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata 7080
actacgatac gggagggcct accatctggc ccagtgctg caatgatacc gcgagaccca 7140
cgctcaccgg ctccagatct atcagcaata aaccagccag ccggaagggc cgagcgcaga 7200
agtggctcctg caactttatc cgctccatc cagtctatta attggtgccg ggaagctaga 7260
gtaagtagtt cgccagttaa tagtttgccg aacgttggtg ccattgctac aggcacgtg 7320
gtgtcacgct cgtcgttttg tatggcttca ttcagctccg gttcccaacg atcaaggcga 7380
gttacatgat ccccatgtt gtgcaaaaaa gcggttagct ccttcgggtc tccgatcgtt 7440
gtcagaagta agttggccgc agtggtatca ctcatgggta tggcagcact gcataattct 7500
cttactgtca tgccatccgt aagatgcttt tctgtgactg gtgagtactc aaccaagtca 7560
ttctgagaat agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat acgggataat 7620
accgcgccac atagcagaac tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga 7680
aaactctcaa ggatcttacc gctgttgaga tccagttcga tgtaaccac tcgtgcaccc 7740
aactgatctt cagcatcttt tactttcacc agcgtttctg ggtgagcaaa aacaggaagg 7800
caaatgccc caaaaaaggg aataagggcg acacggaaat gttgaatact catactcttc 7860
ctttttcaat attattgaag catttatcag ggttattgtc tcatgagcgg atacatattt 7920
gaatgtattt agaaaaataa acaaataggg gttccgcgca catttccccg aaaagtgcc 7980
cctgacgtc 7989

```

<210> 113
 <211> 53
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 113
 gtcactcgag gactcggctg actgaaaatg agacatatta tctgccacgg acc 53

<210> 114
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 114
 cgagatcgat cacctccgtt acaagggttg gcatag 36

<210> 115
 <211> 37
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 115
 catgaagatc tggaagggtg tgaggtacga tgagacc 37

<210> 116
 <211> 51
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 116
 gcgacttaag cagtcagctg agacagcaag acacttgctt gatccaaatc c 51

-129-

<210> 117
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 117
cacgaattcg tcagcgcttc tcgtcgcgtc caagaccc

38

<210> 118
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 118
caccgccggg aggcggcggc gacggggacg gg

32